# CARBOHYDRATES, LIPIDS AND BIOMARKERS OF TRADITIONAL AND EMERGING CARDIOMETABOLIC RISK FACTORS

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**Abstract**

Aim of this PhD thesis was to investigate whether carbohydrates (CHO) or fats, and in particular saturated fats (SFA) have differential effects on cardiometabolic (CM) risk by examining potential links between these macronutrients and surrogate markers of CM risk. This broad aim was approached by two studies. The first study undertook a secondary analysis of the National Diet and Nutrition Survey Rolling Programme 2008-2014 dataset, a cross-sectional survey. The second study built on the findings from Study 1 and was a randomised parallel pilot feasibility study. Data were collected at three time points over the course of eight weeks, at baseline, at interim point and at endpoint.

In the secondary analysis of study 1 (chapter 2), undertaken using the complex samples module in SPSS 25, data (n=2217) was stratified by gender, age group and BMI group. Data was initially analysed for differences in dietary intake of carbohydrates and fats between those that did or did not present with metabolic syndrome (MetS) using General Linear Models. There were very few significant differences between groups. When those with and without MetS were divided into quartiles of intake of carbohydrates (total CHO, starch and sugar) and fat (total, SFA), in most strata (analysed via Logistic Regression) there were increased odds of presenting with MetS in those consuming the highest amount of CHO or the lowest amount of SFA (all $p<.05$).

The second study (chapter 3) was designed to focus more on these apparent links by randomising participants (n=10; 80% male) to either a diet with the aim to increase their CHO intake to ≥50% total energy (the Eatwell Guide (EWG)), or to drastically reduced their CHO intake to ≤50g/d by following a low-carbohydrate, high-fat diet (LCHF). Participants were supplied with guidelines for their allocated diet and given two-week sample meal plans and recipes tailored to their personal preferences were feasible within the remit of their diet. In addition to examining risk markers that are commonly included in such interventions, the trial also investigated the impact on adiposity proxies, food cravings, moderate-to-vigorous physical activity and a number of biomarkers that have emerged as potential CM risk markers in recent years. This included the hepatokine fibrogen growth factor 21 (FGF21). Furthermore, the second study scrutinised the food-groups, carbohydrates and subtypes of SFA that contributed to participants' intake. The findings showed that for some markers, such as sdLDL-C the impact was unambiguous; all participants in the LCHF group presented with significantly decreased sdLDL-C concentrations (up to -34%) and 80% of participants in the EWG group with increased concentrations (up to +15%) ($p=.008$ for diet*time). Overall, insulin concentrations decreased by 45% in the LCHF and 12% in the EWG group

and all LCHF participants presented with reduced insulin concentrations by up to 59% at endpoint but only some EWG did so (by up to 35%) ($p$=.002 for diet*time). For FGF21 only the effect of time was significant ($p$=.044) and overall concentrations declined in the LCHF and increased in the EWG group. In some LCHF participants concentrations of significant CM risk markers were at their lowest with maximum SFA intake with the majority of SFA derived from meat & meat products and dairy.

Overall, the two studies showed that there is scope for further research to investigate the differential effects of different food groups that are either rich in CHO or fats/SFA under consideration of the food matrix. Furthermore, the mechanisms and causes behind the inter-individual responses warrant further investigation to give more tailored and personalised nutrition advice for primary and secondary prevention of CM diseases in the future.

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**Statement of candidate's individual contributions**

NDNS analysis (Study 1)

The NDNS Rolling Programme is an annual survey to assess the dietary intake, nutritional status and lifestyle of the UK free-living population aged ≥1.5 years. The data are freely available to researchers on application and accessible from the UK Data Service. The candidate downloaded and merged the data sets and undertook all secondary analysis.

CALIBER (Study 2)

The candidate and ID were responsible for designing the original study. The candidate was responsible for writing and submitting the original ethics application to Liverpool John Moores University Research Ethics Committee. Participants were recruited by the candidate (TH) and DM. IG, the candidate (TH) and KE undertook the phlebotomy. The candidate (TH) and DM had shared responsibility for data collection and the processing of the blood samples in the CALIBER study. The candidate (TH) had main responsibility for measurement (shared with DM) and sole responsibility for analysing the following variables: systolic blood pressure, diastolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, plasma triglycerides, apolipoprotein B, small dense LDL cholesterol, fasting plasma glucose, hydroxybutyrate, the food frequency questionnaire, the four-day food diaries, the Recent Physical Activity questionnaire, the GT9X accelerometer data, the UK Food Cravings Inventory, height, weight, waist circumference, neck circumference, fat mass and visceral adipose tissue, the adverse events interviews and the final interview on the experiences with the allocated diets. The candidate (TH) wrote the study's dietary guidance notes and designed the meal plans for each participant and sourced the recipes suggested to the participants during the intervention.

The candidate was responsible for data analysis included within this thesis as well as writing of this thesis.

DM undertook the FGF21, adiponectin, leptin and insulin analysis and RW undertook the analysis for LDL-C and apoA1.

**Publications and communications**

This thesis has resulted in the following publications and conference communications:

**Publications with the candidate as first author:**

**Harrison, T**., McCullough, D., Lane, K., Boddy, L., Stewart, C., Enright, K., Amirabdollahian, F. Schmidt, M. and Davies, I. (2018) *Dietary carbohydrate intake, visceral adipose tissue and associated markers of cardiometabolic risk.* Nutrition Society Summer Meeting, 2018 – Getting the energy balance right, Leeds, 10 Jul 2018 - 12 Jul 2018. Proceedings of the Nutrition Society (2018). Cambridge University Press. 77

**Harrison, T.,** McCullough, D., Lane, K., Boddy, L., Stewart, C., Enright, K., Amirabdollahian, F. Schmidt, M. and Davies, I. (2018) *The association between dietary macronutrient intake and fibrogen growth factor 21 in a sample of white UK adults with elevated cardiometabolic risk markers.* Nutrition Society Summer Meeting, 2018 – Getting the energy balance right, Leeds, 10 Jul 2018 - 12 Jul 2018. Proceedings of the Nutrition Society (2018). Cambridge University Press 77

**Harrison, T**., Lane, K., Boddy, L., Amirabdollahian, F., Davies, I. (2017) *Macronutrient intake and prevalence of markers of metabolic syndrome in white UK adult males in the National Diet and Nutrition Survey Rolling Programme 2008 – 2014*. Nutrition Society Summer Meeting, 2017 – Nutrition in metropolitan areas, London, 10 Jul 2017 - 12 Jul 2017. Proceedings of the Nutrition Society (2017). Cambridge University Press. 76

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**Conference communications:**

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**Harrison, T**., Lane, K., Boddy, L., Amirabdollahian, F., Davies, I. (2018) Dietary intake and cardiometabolic risk in the National Diet and Nutrition Survey 2008-2014. Poster presentation, *Women in Research*, Liverpool, UK, Liverpool John Moores University,

**Harrison, T**., Lane, K., Boddy, L., Amirabdollahian, F., Davies, I. (2018) Dietary intake and cardiometabolic risk in the National Diet and Nutrition Survey 2008-2014. Poster presentation, *Early Careers Researchers in Food Networking Event*, Birmingham The Knowledge Transfer Network

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**Harrison, T**., Lane, K., Boddy, L., Amirabdollahian, F., Davies, I. (2017) Personalised nutritional approaches to ameliorate cardiometabolic risk in normal-weight obesity (2017). Poster presentation. *CPH PhD symposium*, Liverpool, UK, Liverpool John Moores University

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**Harrison, T., Davies, I.** (2015) Krill oil as sustainable source of omega-3 PUFA for dyslipidaemia. Oral communication, *Institute of Food Science and Technology North Young Scientist 2015 competition*, Manchester, UK, Manchester Metropolitan University

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**Abbreviations**

| | |
|---|---|
| %E | Percent energy |
| %TE | Percent total energy |
| ADPN | Adiponectin |
| AOR | Adjusted odds ratio |
| apoA1 | apolipoprotein A-1 |
| apoB | apolipoprotein B |
| apoB/apoA1 | apolipoprotein B/apolipoprotein A-1 ratio |
| BIA | Bioelectrical impedance analysis |
| BMI | Body mass index |
| BP | Blood pressure |
| CAD | Coronary artery disease |
| CALIBER | Carbohydrates, lipids and biomarkers of traditional and emerging cardiometabolic risk factors |
| CAM | Cellular adhesion molecule |
| CHO | Carbohydrate |
| CM | Cardiometabolic |
| CMD | Cardiometabolic disease |
| CV | Cardiovascular |
| CVD | Cardiovascular disease |
| DBP | Diastolic blood pressure |
| DXA | Dual-energy x-ray absorptiometry |
| EWG | Eatwell Guide |
| FBG | Fasting plasma glucose |
| FD | Food diary |
| FETA | Food Frequency Questionnaire European Prospective Investigation into Cancer and Nutrition Tool for Analysis |
| FFQ | Food Frequency Questionnaire |
| FCI-UK | UK Food Cravings Inventory |
| FGF21 | Fibroblast growth factor 21 |
| FM | Fat mass |
| GLUC | Glucose |
| Hb1Ac | Glycated haemoglobin |
| HC | High-carbohydrate |
| HDL-C | High-density lipoprotein cholesterol |
| HOMA2-IR | Homeostasis Model Assessment (of insulin resistance) |
| ICF | Informed Consent Form |
| INS | Insulin |
| ITT | Intention to treat analysis |
| IR | Insulin resistance |
| KHANES | Korean National Health and Nutrition Examination Survey |
| LCHF | Low-carbohydrate, high-fat |
| LCSFA | Long-chain saturated fatty acid |
| LDL-C | Low-density lipoprotein cholesterol |
| LDLR | Low-density lipoprotein receptor |
| LEP | Leptin |
| LF | Low-fat |
| LJMU REC | Liverpool John Moores University Research Ethics Committee |
| MA | Meta-analysis |
| MCSFA | Medium-chain saturated fatty acid |
| METs | Metabolic syndrome |
| MFP | Main food provider |

| | |
|---|---|
| MUFA | Mono-unsaturated fatty acid |
| MVPA | Moderate-to-vigorous physical activity |
| NCD | Non-communicable disease |
| NDNS RP | National Diet and Nutrition Survey Rolling Programme |
| NHANES | National Health and Nutrition Examination Survey |
| NO | Nitric oxide |
| OR | Odds ratio |
| PA | Physical activity |
| PAD | Peripheral artery disease |
| PCA | Principle Component Analysis |
| PCS | Prospective cohort study |
| PHE | Public Health England |
| PIS | Participant Information Sheet |
| PP | Per protocol analysis |
| PUFA | Polyunsaturated fatty acid |
| RCT | Randomised-controlled trial |
| ROS | Reactive oxygen species |
| RPAQ | Recent Physical Activity Questionnaire |
| SACN | Scientific Advisory Committee on Nutrition |
| SBP | Systolic blood pressure |
| sdLDL-C | Small dense low-density lipoprotein cholesterol |
| SFA | Saturated fatty acids |
| SR | Systematic review |
| SRep | Scavenger receptor |
| STAR | Total starch |
| SUG | Total sugars |
| T1DM | Type 1 diabetes mellitus |
| T2DM | Type 2 diabetes mellitus |
| TG | Triglycerides |
| TG/HDL-C | Triglyceride/High-density lipoprotein cholesterol |
| UK | United Kingdom |
| VAT | Visceral adipose tissue |
| VLDL | Very-low-density lipoprotein |
| VSMC | Vascular smooth muscle cell |
| WC | Waist circumference |

# Chapter 1 - Introduction and background to the research problem

## 1.1 The impact of cardiometabolic diseases on mobidity and mortality

Cardiometabolic diseases (CMDs), which include cardiovascular diseases (CVD) and type 2 diabetes mellitus (T2DM) contribute globally to over 50% of deaths from non-communicable diseases (NCDs) (Benziger et al., 2016; WHO, 2014). Premature mortality rates from CMDs have been declining in high-income countries (including the UK), yet they considerably increase morbidity, negatively affect quality of life in later years (Bennett et al., 2018; Benziger et al., 2016; James et al., 2018) and cause considerable costs for health care systems, the wider economy and individuals (Wilkins et al., 2017; Devaux et al., 2019; Einarson et al., 2018; Hex et al., 2012; Jan et al., 2018).

## 1.2 Clinical markers of cardiometabolic risk

### 1.2.1 Blood lipids and blood lipid ratios

A wide range of biomarkers potentially impacted by carbohydrates (CHO) and saturated fatty acids (SFA) have been identified as surrogate markers of CM risk. Of these, blood lipids are amongst those which have been most thoroughly investigated; these include low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG). The potential association of various types of lipoproteins has been the subject of animated debate for decades (addressed in further detail in chapter 3.1.2.2). Knowledge of which marker might pose greater risk is constantly evolving with the advance of biomedical and biochemical methods and equipment. Historically, high concentrations of HDL-C and low concentrations of LDL-C were deemed beneficial for CM health and inversely related to the occurrence of CMDs (Nicholls and Nelson, 2019; van der Steeg et al., 2006). A decrease in LDL-C levels remains the primary target for CVD prevention (Catapano et al., 2016). Elevated TG levels (hypertriglyceridaemia) are known to be an independent risk factor of CVD (Austin et al., 1991; Budoff, 2016; Rosenson et al., 2014) They are indicative of TG-rich very-low density lipoprotein (VLDL) and chylomicron remnants, which are also considered atherogenic (Lawler et al., 2017; Twickler et al., 2005). Furthermore, TG is a good predictor of small dense LDL lipoprotein (sdLDL) (Tsimihodimos et al., 2007), the most atherogenic LDL (see chapter 3.1.2.2). For those classified at intermediate risk of CVD assessment of TG concentrations can be used to further determine risk category (Catapano et al., 2016). Concentrations tend to be higher in men but increase with age in both men and women (Tenenbaum et al., 2014).

### 1.2.2 Blood lipid ratios

More recent evidence has shown that calculating the ratios of selected lipids are useful if not superior tool for CM risk prediction than single markers in isolation (Catapano et al., 2016; Millán et al., 2009). The TG/HDL ratio demonstrated a high degree of discriminatory power to identify both men and women with MetS (Gasevic et al., 2014), and a higher TG/HDL-C ratio was associated with increased risk of presenting with atherogenic dyslipidaemia (Quispe et al., 2015). In a white population, the TG/HDL ratio was also deemed an acceptable predictor of insulin resistance (IR) (Kim-Dorner et al., 2010). A TG/HDL ratio of >3.5 has been associated with increased CVD risk in men and women (Upadhyay, 2015).

### 1.2.3 Fasting blood glucose

Fasting blood glucose (FBG) levels are used as a biomarker of CM health to check whether glucose is cleared from the circulation in sufficient time. Levels of FBG determine whether an individual would be categorised as metabolically healthy or impaired (pre-diabetic or diabetic) and are a primary treatment goal in T2DM (NICE, 2012; NICE, 2015). Its levels differ with age, gender and ethnicity (White et al., 2015) and can be affected by acute illness or stress (Krhač and Lovrenčić, 2019). Furthermore, there is a positive association between FBP and risk of CV morbidity and mortality (Coutinho et al., 1999). This is because hyperglycaemia affects endothelial and smooth muscle cells in blood vessels leading to a reduction in blood vessel diameter and consequently to hypertension (Jackson et al., 2016).

### 1.2.4 Hypertension

Hypertension (defined as SBP ≥140 mmHg or DBP ≥90 mmHg) and pre-hypertension (defined as SBP ≥135 mmHg or DBP ≥85 mmHg) are amongst the highest risk factors for cardiovascular morbidity and mortality (Jeemon et al., 2017). This is independent of age, gender and ethnicity (Stamler et al., 1993), although the prevalence of hypertension increases with age (Wu et al., 2015). Systolic blood pressure appears to have stronger links with CVD risk than diastolic blood pressure (Stamler et al., 1993).

## 1.3 Aetiology of atherosclerosis

Atherosclerosis is a condition in which the arteries experience an increasing promotion of plaque on their inner walls, which can eventually lead to hardening and narrowing of the vessels (Brown et al., 2017). It is a chronic disease, which develops silently over many decades (Huff et al., 2016). First signs of atherosclerosis in form of fatty streaks, which adhere to the initial wall of the blood vessels, already occur in children and adolescents (Hong, 2010; Mendis et al., 2005; Seidman et al., 2014). It mainly affects the aorta and

carotid arteries, but can also present in medium-sized arteries (Rajamani and Fisher, 2017). Atherosclerosis is a serious condition, which, if not halted or even reversed, can lead to CVDs, such as coronary artery disease (CAD), cerebrovascular disease and peripheral artery disease (PAD) (Frayn et al., 2019; Wang and Butany, 2017), combined one of the leading causes of global morbidity and mortality (Benzinger et al., 2016; WHO, 2014).

Risk factors for atherosclerosis include hypercholesteraemia/dyslipidaemia (Hurtubise et al., 2016), hyperglycaemia (Kanter et al., 2007; Libby, 2013), hypertension (Hurtubise et al., 2016), obesity (Burke et al., 2008), smoking (McEvoy et al., 2015), increasing age (Head et al., 2017), male gender or postmenopausal female (Libby, 2013) and genetic factors (Brown et al., 2017; Kanter et al., 2007). Apart from the latter three, all of these risk factors are modifiable via diet or medication (Stewart et al., 2017).

### 1.3.1 Stages of atherosclerosis development and progression

Traditionally, atherosclerosis was deemed to be a disease of hypercholesterolemia/hyperlipidaemia alone (Libby, 2013) as in its earliest stages it is mainly free cholesterol and fatty acids that can be found in the fatty streaks (Rafieian-Kopaei et al., 2014; Seidman et al., 2014). However, more recent research has elucidated that the beginnings and the progression of atherosclerosis are marked by a complex interplay of several factors (Figure 1.1). These include, but are not limited to, endothelial dysfunction, lipid metabolism, profilation and migration of vascular smooth muscle cells (VSMC) and innate immune response (Wang et al., 2012). The locations most vulnerable to plaque development are branch points where blood flow might be high-pressured, turbulent or reversing and where lipoproteins and blood monocytes might therefore have a prolonged residence time (Libby, 2013; Schwarzt and Valente, 1994; Wang and Butany, 2017).

#### 1.3.1.1 Endothelial dysfunction

At atherosclerosis-prone sites, the cells of the endothelium re-arrange themselves in response to the disturbed flow, consequently weakening the structure of the arterial intima, the middle layer of the endothelium (Libby, 2013). This initiates a cascade of events. Firstly, the endothelium becomes more permeable; secondly, it secretes less of the vasodilatory nitric oxide (NO), the latter exerting anti-inflammatory and anti-prolifiratory properties (Rafieian-Kopaei et al., 2014). The anti-inflammatory function of NO stems from its ability to inhibit leukocytes and macrophages from attaching to the endothelial wall causing injury (and necessitating a response from the innate immune system). Its anti-prolifiratory function stops VSMCs, which make up the middle layer of the artery wall (tunica media), from infiltrating the intima during disease progression (Libby, 2013; Seidman et al., 2014; Wang et al., 2012).

As a first step in atherosclerosis development, the permeable endothelial wall is more likely to be infiltrated by circulating cholesterol-rich, apolipoprotein B (ApoB)-containing lipoproteins, mainly LDL; these accumulate and are retained within the intima (Moore et al., 2013; Tabas et al., 2007). More recently, the perception of atherosclerosis as a *response-to-injury* disease (Brown et al., 2017; Gui et al., 2012) has therefore shifted to one of a *response-to-retention* (Tabas et al., 2007).

#### 1.3.1.2 Lipid retention as initial trigger of atherosclerosis

Nascent LDL is taken up by tissue through the apoB-binding cell surface protein LDL receptor (LDLR) (Seidman et al., 2014; Schneider, 2016). In hypercholesteraemia expression of LDLR is downregulated to prevent lipid accumulation in cell tissue; this also serves to counteract the effects of increased free cholesterol within the cell matrix, which elicits toxic effects (Huff et al., 2016; Moore et al., 2013). In atherosclerosis, there are a number of alternative pathways allowing for lipids to enter the cell matrix nonetheless. The majority of LDL particles undergo modification mainly via oxidation and acetylation but other enzymatic and non-enzymatic pathways are also utilised (Alique et al., 2015; Huff et al., 2016). In diabetics, LDL can also undergo modification via non-enzymatic glycation (Kanter et al., 2007; Seidman et al., 2014).

Oxidised LDL is recognised by scavenger receptors (SRep), also called pattern recognition receptors (Schneider, 2016). Some of these are activated by blood glucose (Kanter et al., 2007), which demonstrates that glycaemic control, especially in diabetes (see below) is an important factor in atherosclerosis management and prevention. Other processes of modified LDL uptake include those which are endocytic, phagocytic or nonreceptor mediated. Lipolytic enzymes modify LDL to a form that is recognised and taken up by macrophages through a nonreceptor mediated process (see below). Nascent LDL can also enter macrophages independently of receptors, for example via pinocytosis (Huff et al., 2016; Moore et al., 2013). However, as Moore et al. (2013) have highlighted, many of these modifications have been identified in vitro and it remains unclear whether (all of) the same pathways are involved in human atherosclerosis.

Whether it is mainly the amount of LDL-C in the circulation or the number and size of LDL particles has been a subject of debate in recent years (Diffenderfer and Schaefer, 2014; Schwarzt and Valente, 1994; Tabas et al., 2007). This is addressed is further detail in chapter 3 of this thesis. However, as Kanter et al. (2007) highlight that at least in hyperglycaemia, LDL-C concentrations need to exceed a (not specified) threshold to contribute to atherosclerosis.

#### 1.3.1.3 Innate immune response and pro-inflammatory factors

Both the increased permeability of the endothelium per se and the presence of nascent and modified LDL within the intima triggers an innate immune response. Circulating monocytes, which occur in greater numbers in hypercholesteraemia (Huff et al., 2016), are summoned to the intima via chemokines (Moore and Tabas, 2011). Downregulated expression of NO (see above) results in upregulation of cellular adhesion molecules (CAM) on the endothelial surface to which the monocytes attach (Moore et al., 2013). They subsequently enter the intimal matrix and can become activated to pro-inflammatory macrophages, which are mainly of type M1 (Huff et al., 2016; Moore et al., 2013).

Both the uptake and accumulation of lipoproteins and monocytes-turned-macrophages trigger a positive feedback loop. The action of reactive oxygen species (ROS), a by-product of lipid modification, further increase endothelial permeability, which consequently permits yet more lipoproteins to enter the intima (Wang et al., 2012). Modified LDL also degrade less easily prolonging their residential time within the intima (Wang et al., 2012).

#### 1.3.1.4 The development of foam cells

Nascent and modified lipoproteins are hydrolysed into free cholesterol and fatty acids after engulfment by the macrophages and the resulting free cholesterol can be toxic to the cell (Moore et al., 2013). At this stage the development of foam cells (containing re-esterified cholesteryl fatty acid esters), characterised through their white, foamy appearance and contributing to the fatty streak, can be observed (Moore and Tabas, 2011). As the accumulation of nascent and modified lipids and the hydrolysis of LDL to produce free (toxic) cholesterol is perceived by the foam cell as a danger, it recruits inflammatory cells to its aid. These also include types of a pro-inflammatory nature (Seidman et al., 2014). This in turn triggers yet another positive feedback loop through the recruitment and activation of increasing numbers of circulating monocytes to M1 macrophages (Seidman et al, 2014). The process of foam cell formation is accelerated. Macrophages accumulating in the intima lose the ability migrate from the endothelial matrix, thus aiding the built-up of lesions and progression of atherosclerosis further (Moore et al., 2013). Whilst the lesions (fatty streaks) in early atherosclerosis seem to be composed mainly of lipids, the atheroma on advancing and advanced atherosclerosis are characterised by a large number of VSMCs (Dubland and Francis, 2016).

#### 1.3.1.5 The role of vascular smooth muscle cells in the progression of atherosclerosis

The upregulation of CAM in the endothelium also causes VSMC to migrate to the site of the injury as an innate immune response, where they proliferate (also encouraged by the

downregulation of NO (see above)) and eventually thicken the endothelial wall (Wang et al., 2012). At this point, another positive feedback loop crucial for the progression of atherosclerosis is triggered. Vascular smooth muscle cells in the intima also express adhesion molecules and cytokines attracting more monocytes to the site. Furthermore, they induce further migration and proliferation of VSMC into the intima and prolonged endothelial dysfunction (Wang et al., 2012). This process leads to the evolvement of fatty streaks into transitional lesions in progressing atherosclerosis (Libby, 2013; Schwarzt and Valente, 1994).

#### 1.3.1.6 Further progression of atherosclerosis

If not managed by lifestyle changes or medication the fatty streaks observed from an early age mature into atherosclerotic plaques. Through the self-perpetuating mechanisms described earlier, these eventually evolve into stable or unstable/vulnerable plaques obstructing the artery. Eventually, these can either rupture (unstable plaques) leading to for example myocardial infarctions or stroke, or present as thrombotic occlusions (stable plaques) leading to for example stable angina (Seidman et al., 2014).

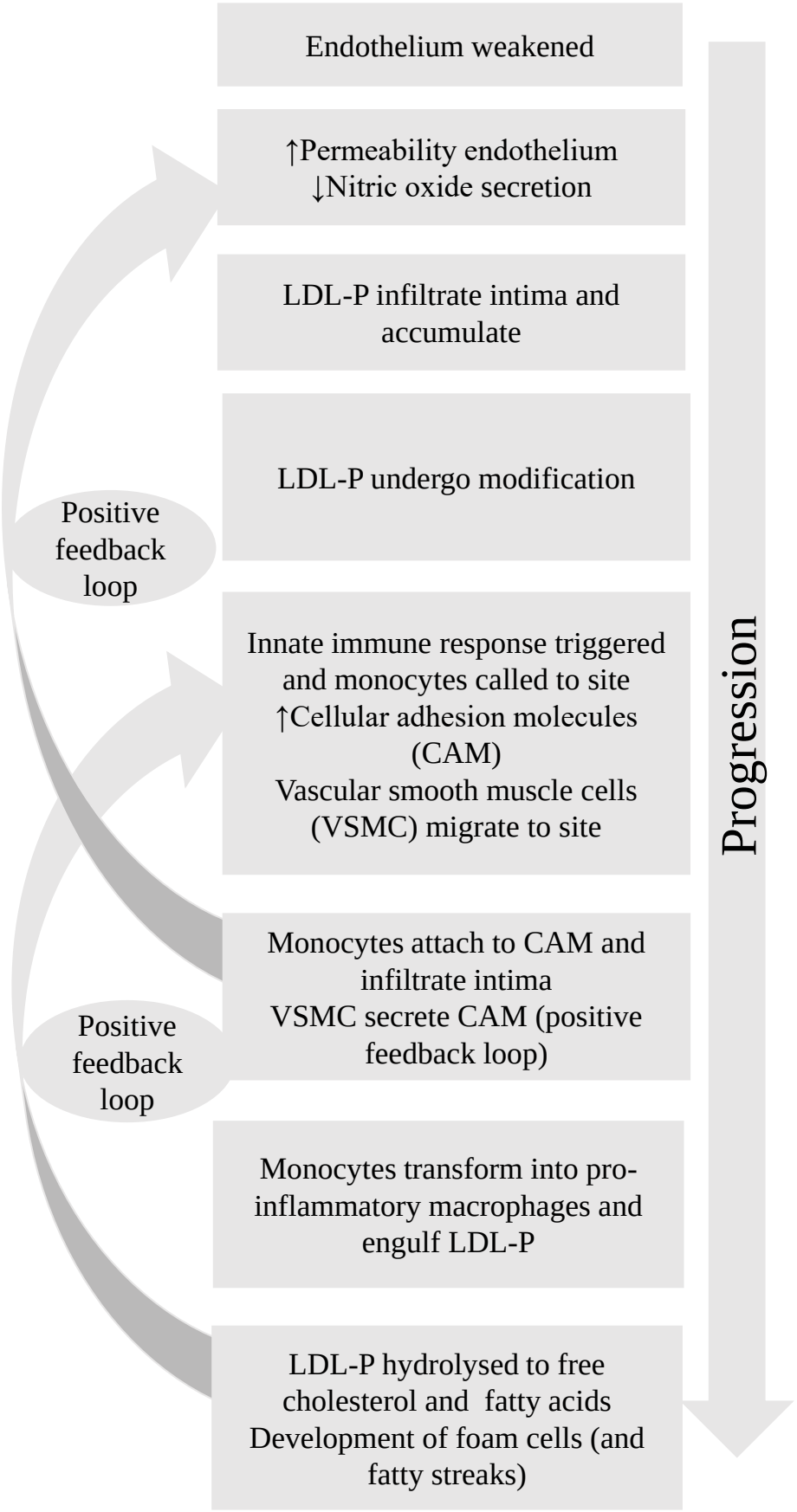


Figure 1.1 Pathogenesis of atherosclerosis

#### 1.3.1.7 The role of high-density lipoprotein in atherosclerosis

Excessive lipid uptake into the macrophages infiltrating the intima in progressing atherosclerosis leads to the downregulation of processes that in a healthy individual lead to removal of (excess) cellular cholesterol via HDL (Huff et al., 2016; Moore et al., 2013; Yvan-Charet et al., 2010). This is under metabolically healthy circumstances one of the beneficial roles of HDL as the removal of free cholesterol prevents or aids in the reversal of the formation of foam cells and subsequent inflammation (Francis, 2016; Moore and Tabas, 2011; Yvan-Charet et al., 2010). Other roles of HDL include the repair of dysfunctional endothelial cells, the upregulation of NO secretion, the downregulation of CAM expression and of macrophagal secretion of pro-inflammatory cytokines (Frances, 2010). Another reason why HDL has been attributed anti-atherogenic properties is that is it the preferred substrate for oxidation in place of LDL, thus potentially limiting the amount of LDL modified in this manner (Frances, 2016). However, this oxidation can also render HDL ineffective, devoiding it of its anti-atherogenic properties (Frances, 2016; Huff et al., 2016). A number of other factors affect HDL function, such as presence of diabetes mellitus and systemic inflammation (Frances, 2016). In T2DM patients, HDL has been found to be less effective in upregulating NO and stimulating endothelial repair mechanisms (Sorrentino et al., 2010).

### 1.3.2 Other factors impacting development and progression of atherosclerosis

#### 1.3.2.1 Hyperglycaemia

Hyperglycaemia has been found to be an independent contributor to atherosclerosis (Libby, 2013). It occurs both in type 1 and type 2 diabetes mellitus (T1DM and T2DM; insulin-dependent and insulin-independent types) and in T2DM is more often than not accompanied by dyslipidaemia (Kanter et al., 2007). Elevated fasting blood glucose concentrations are an independent risk factor for changes in the endothelial phenotype via modification of proteins contained within the cell matrix. This happens through the binding of these proteins to advanced glycation end products (AGE), which increases endothelial permeability (Giacco and Brownlee, 2010) and production of ROS (Theodorou and Boon, 2018). Further mechanisms in which hyperglycaemia can contribute to tissue damage have been reviewed by Giacco and Brownlee (2010).

Due to higher expression of CAM in endothelial cells (Kanter et al., 2007) diabetic patients present with an altered arterial matrix, which might be reason for higher risk of CAD compared to non-diabetic individuals with similar LDL concentrations (Tabas et al., 2007). Whilst in T2DM dyslipidaemia appears to be a driving force in atherosclerosis development and progression, in T1DM hyperglycaemia and associated accelerated progression of lesions

seem to be the major factor (Kanter et al., 2007). In T2DM hyperglycaemia and dyslipidaemia work synergistically accelerating development and progression of plaque (Kanter et al, 2007).

#### 1.3.2.2 Insulin resistance

Insulin resistance (IR) (see chapter 3.1.2.3 for in-depth discussion) increases the risk of CAD even without hyperglycaemia (Bornfeldt and Tabas, 2011). Endothelial cell, VSMCs and macrophages have insulin receptors and their insulin receptor-mediated signalling pathways are downregulated in hyperinsulinaemia (Bornfeldt and Tabas, 2011). This can lead to downregulation of NO and upregulation of CAMs. Furthermore, insulin stimulates VSMC profileration (DeFronzo, 2010). Therefore, IR can independently trigger the mechanisms attributed to atherosclerosis development and progression as previously described.

#### 1.3.2.3 Hypertension

Although hypertension is an independent risk factor of atherosclerosis, it can worsen the disease synergistically with dyslipidaemia, for example in those presenting with MetS (Hurtubise et al., 2016). Hypertension puts pressure on the endothelium, therefore potentially damaging the matrix and consequently contributing to its permeability, which is partially due to the development of free radicals in the endothelial wall (Alexander, 1995; Rafieian-Kopaei et al., 2014). In addition, the increased pressure can trigger VSMC migration and proliferation with the aim to strengthen the endothelial wall and reduce injury (Alexander, 1995). Blood pressure lowering has therefore been found to decrease endothelial permeability to LDL (Tabas et al., 2007).

#### 1.3.2.1.4 Metabolic syndrome

Metabolic syndrome (MetS) is a cluster of cardiometabolic risk factors including hypertriglyceridaemia, hyperglycaemia, central obesity, hypertension or reduced HDL-C concentrations. A MetS diagnosis is made once an individual presents with three out of five of these clinical markers. A full definition of MetS is included in chapter 2.2.4.

It is important to note that in metabolic syndrome several independent factor contributing in their own right to atherosclerosis development and progression are combined. The resulting synergy has accelerating effects as highlighted earlier. Hyperglycaemia plus dyslipidaemia or dyslipidaemia plus hypertension are examples of such synergies. In an individual presenting with MetS all three of these can be present, making an earlier diagnosis and management of individual components of MetS the more urgent.

### 1.3.3 Limitations in atherosclerosis research

One serious limitation in our understanding of atherosclerosis is that the majority of findings stem from in vitro and animal models, rather than human models. This somewhat limits the potential applicability of findings as these do not fully reflect the complex environment of the endothelium and all potential factors involved in atherosclerotic lesion evolvement and progression (Kanter et al., 2007; Moore and Tabas, 2011; Moroni et al., 2019; Tabas et al., 2007; Theodorou and Boon, 2018). As Huff et al. (2016) highlighted, in animal models of the disease the induction of hypercholesterolaemia (by genetic or dietary means) is a prerequisite of producing atherosclerotic lesions. However, the models do for example not capture the importance of endothelial dysfunction as a vital trigger to monocyte adhesion to and macrophage infiltration of the intima as early step of atherosclerosis development (Theodorou and Boon, 2018). Likewise, animal models do not necessarily reflect the human diabetic phenotype accurately enough (Giacco and Brownlee, 2010). Furthermore, there might be differences in the cell origins of foam cells and atherosclerotic plaques as Dubland and Francis (2016) have found in their work on the VSMCs in atherosclerosis. Whilst in animal models the macrophages contained in progressing lesions seem to be monocyte-derived, in humans their origin appears to be VSCMs, which might impact how disease management and prevention need to be approached (Dubland and Francis, 2016).

### 1.3.4 The role of diet in the development, prevention and management of atherosclerosis with focus on carbohydrates and fats

As dietary intake has been identified as one of the modifiable risk factors for atherosclerosis and CMD (Deghgan et al., 2017; GBD 2017 Diet Collaborators; Grashuber et al., 2016; Micha et al., 2017; Schwingshackl et al., 2018a; Schwingshackl et al., 2018b), the mechanistic effects of individual nutrients, including dietary carbohydrates (CHO) and fats, have been closely scrutinised. As with the elucidation of the general mechanisms of atherosclerosis development the majority of studies investigating mechanistic effects of macronutrients on this have been undertaken in vitro and in animal models (Fernandez and West, 2005; Zhou et al., 2015 REF)

#### 1.3.4.1 The mechanistic links between dietary fats and low-density lipoprotein

In the 1950s Ancel Keys commenced his *Seven Countries study*, which would provide data to establish what would become known as his *lipid hypothesis*. This postulated that the consumption of dietary fats in general and SFA in particular leads to increased risk of CVD morbidity and mortality (Bier, 2016). Of special interest in this context is the intake of different subtypes of dietary fats and the impact that these have on LDL cholesterol due to the role of LDL in atherosclerosis as outlined above.

Both CHO and dietary fats can serve as substrates to supply the human body with energy with CHO being the preferred substrate, and although SFA can also be oxidised to provide energy (Melzer, 2011), the presence of sufficient CHO for energy metabolism is a limiting factor in this. Any SFAs, which cannot be oxidised, have a prolonged residence time in the blood circulation. Saturated fatty acids downregulate LDLR so that LDL clearance from the circulation is reduced and consequently LDL-C concentrations increase, especially with intake of lauric (12:0), myristic (14:0) and palmitic (16:0) saturated fatty acids (Fernandez and West, 2005; Mustad et al., 1997; Woollett et al., 1992). To the contrary, polyunsaturated fatty acids (PUFA), especially omega-3 (n3) PUFA, and to a lesser degree mono-unsaturated fatty acids (MUFA), upregulate LDLR (Fernandez and West, 2005; Zhou et al., 2015) and hence accelerate LDL clearance. It it is for this reason that dietary guidelines advise that the consumption of PUFAs and MUFAs is preferable to the intake of SFA and that the balance between SFA and (n3)PUFA (a higher n3/SFA ratio) has been highlighted as an important aspect of CMD prevention (Saponaro et al., 2015; Siri-Tarino et al., 2015). Indeed, an animal model found that only when n3PUFAs are consumed in insufficient amounts do SFA raise blood cholesterol (MacDonald-Wicks and Garg, 2004).

#### 1.3.4.2 Dietary carbohydrates and de novo lipogenesis

In this context the source of SFA is an important aspect to consider. Studies have established that in the process of hepatic *de novo lipogenesis* (DNL) plasma SFA (mainly palmitic, but also stearic, lauric and myristic FA) are derived from dietary CHO through conversion pathways (Ruiz-Nuňez et al., 2016). Reduced-CHO diets have been shown to decrease plasma SFA concentrations (Forsythe et al., 2008; Forsythe et al., 2010). High-CHO diets could therefore be significant contributors to plasma fatty acid concentrations; simple sugars, in particular fructose, are more readily utilsed in DNL (Ameer et al., 2014). As the majority of fatty acids derived from DNL are saturated and therefore have the potential to raise LDL-C via downregulation of LDLR, the impact of high-CHO diets, especially in combination with high dietary SFA has to be acknowledged as a potentially high risk factor in CM health and disease. Furthermore, hepatic esterification of DNL-derived lipids gives rise to large triglyceride-rich particles that subsequently get converted to small dense LDL, which are more detrimental to CM health (Siri-Tarino et al., 2015) (see chapter 3.1.2.2). In this context PUFA can exert beneficial effects as their presence decreases DNL (Ameer et al., 2014).

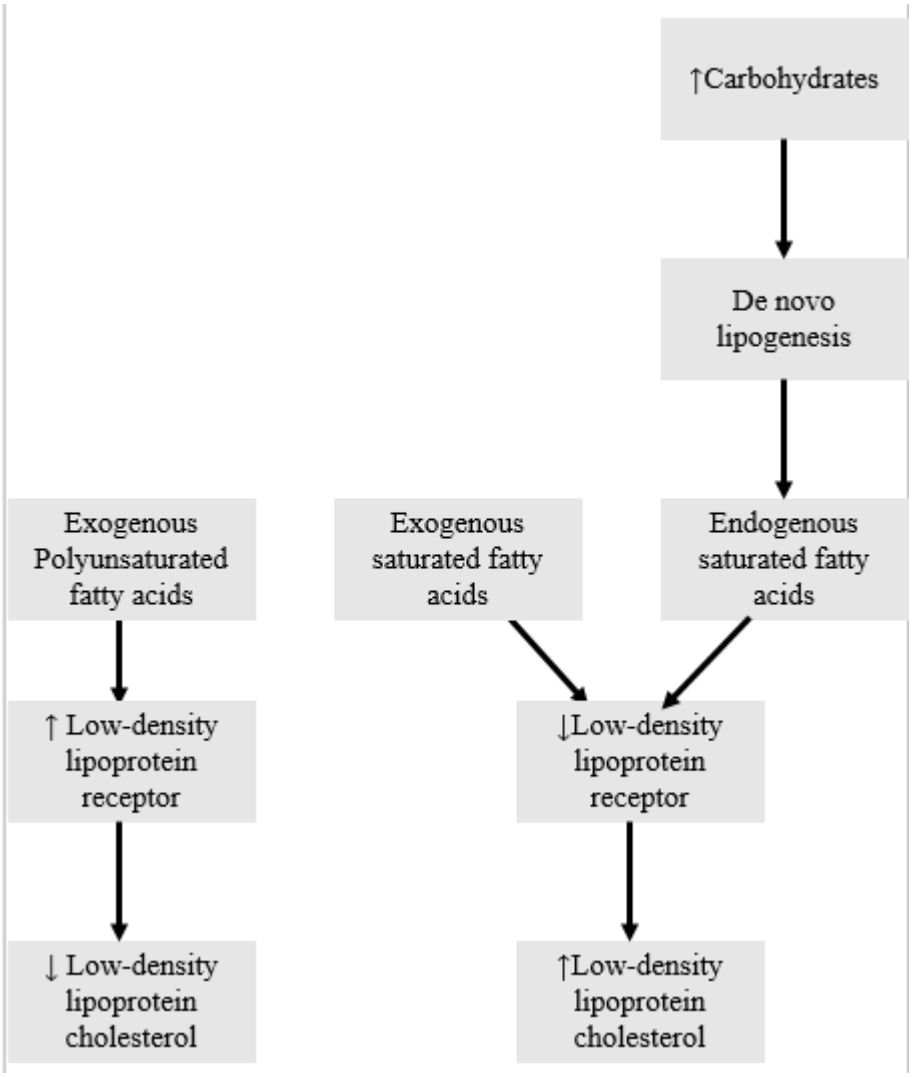


Figure 1.2 Mechanistic effects of dietary carbohydrates, saturated fatty acids and polyunsaturated fatty acids on low-density lipoproteins cholesterol concentrations

### 1.4 The impact of dietary carbohydrates on cardiometabolic disease risk, mobidity and mortality

In 2015 SACN published their report reviewing and synthesising evidence from prospective cohort studies (PCS) and randomised-controlled trials (RCT) on carbohydrates and (CM) health, of which the results were subsequently incorporated into the current UK dietary recommendations (Public Health England, 2016a). Meta-analysis of three cohort studies found moderate evidence of no association between total CHO intake and CVD events. Meta-analyses for other CMD outcomes were not be performed due to scarcity of studies eligible for inclusion. There was limited evidence of no association between total CHO intake and coronary events, and between total CHO intake and stroke incidence. There was also moderate evidence of no association between total CHO intake and incidence of T2DM. The evidence for associations between total sugar intake and coronary events was also moderate and showed no associations between total sugars and coronary events, whereas the

evidence for total sugar intake and incidence of T2DM was limited and also showed no associations. There was moderate evidence of no association between total starch intake and coronary events or incidence of T2DM, and between intake of refined grains and CVD events or incidence of T2DM. The SACN review included only studies conducted post-1990 for the reasons that studies before this time had been assessed in previous governmental reviews. However, as statistical methods and methodologies to conduct systematic reviews and meta-analysis have advanced since then it might have been worthwhile to re-analyse these studies and hence with achieve a larger evidence base to build upon. This might have made the performance of more meta-analyses possible. As there was no evidence found of any associations between total CHO and subgroups of CHO and the outcome measures assessed, SACN concluded that the dietary recommendations for CHO should remain at levels previously advised and set them at ≥50%TE.

Since the SACN report on CHO was published findings from other PCS have emerged contributing to the body of evidence. Dehghan et al. (2017) reported on the results from a multi-national cohort study, the Prospective Urban Rural Epidemiology (PURE), with data from 135,335 participants (35-70 years) from 18 countries enrolled between 2003 and 2013. Median follow-up was 7.4 years. Primary and secondary outcomes included CV morbidity and mortality. Dietary intake was assessed at baseline via a food frequency questionnaire (FFQ), but because of variations in validated country-specific questionnaires, the number of food items ranged from 95 to 250. Participants were divided into quintiles of total CHO intake (%TE). There were no significant differences in risk of CVD morbidity or mortality between quintiles. Sources of CHO were not defined, although other studies based on the PURE data set have examined the associations of CMD with fruit, vegetables and legumes (Miller et al., 2017). Dehghan et al. (2017) also published supplementary data of the main five food sources of macronutrients for each country with their supplemental material. The accumulative contribution to CHO intake by food group was not reported.

Seidelman et al. (2018) conducted a PCS on dietary carbohydrate intake and mortality. The researcher examined the data from 15,428 adults (45–64 years), who were enrolled in the Atherosclerosis Risk in Communities (ARIC) study between 1987 and 1989. Median follow-up was 25 years. The dietary data used in the analysis was collected via FFQ at baseline (visit 1) and 6 years later (Visit 3). For participants developing CMD before visit 3 CHO intake from baseline was used in the analysis. For the other participants, dietary data from visit 1 was used if the time point assessed fell before visit 3. If the analysis included data from visit 3 onwards, the cumulative average from mean CHO intake at visit 1 and at visit 3 was calculated. A U-shaped relationship between CHO intake and CVD mortality was found

with hazard ratios lowest in the 50%TE – 55%TE range. Whilst this study took into account potential changes in CHO intake after 6 years, which was an advantage towards similar studies, no further potential dietary changes were considered. This is important, as a potential change in dietary intake/habits in this time period might have impacted the results differently. The types and source of CHO was not included in the analysis. However, in a modelling exercise substituting CHO with plant-derived proteins decreased hazard ratios for CVD mortality demonstrating that diet quality should be considered as a confounding factor.

### 1.5 The impact of saturated fatty acids on cardiometabolic disease risk, morbidity and mortality

One of the most recent attempts to review the effect of SFAs on CMDs (outcomes, intermediate markers and risk factors) was undertaken by SACN (2018). Whilst the initial request made by the Food Standards Agency (Scotland) (now Food Standards Scotland) (FSS)) included all fats, it was recognised that the review of SFA was most pressing and should be prioritised. Included in the evidence-based were meta-analyses (MA), systematic reviews (SR) and pooled analysis that on focused RCTs and PCSs. Excluded were MAs and SRs of case-control or cross-sectional studies. There was also no re-analysis of trials prior to 1991 when the COMA report was published. For CVDs 17 SRs with or without MAs were identified. There was adequate evidence of no effect of SFA on CVD mortality from RCTs and adequate evidence of no association between SFA and CVD mortality from PCS. There was insufficient evidence from PCS to link SFA to CVD events but adequate evidence from RCTs that reduced SFA intake lowers the number of CVD events. For T2DM, six SRs (four with MAs) were identified. No evidence was found between SFA and T2DM for RCTs and there was adequate evidence from PCS of no association between SFA and T2DM. Based on the overall findings the draft report recommended that the UK reference values for SFA remain unchanged. It was beyond the remit of the SACN review to examine the effect of different subgroups of SFAs and of different foods and food groups that contain these. This might be due to scarcity of SRs and MAs on these (Panth et al., 2018). Future reviews informing dietary guidelines and nutrition policies should ensure that they recognise these potential differences once the evidence base becomes strong enough.

Since the publication of SACN's draft report for public consultation additional findings from a number of PCS have been published that can contribute to the body of evidence.

Dehghan et al. (2017) also reported on the impact of total fats and SFA on CVD morbidity and mortality as discussed previously. They found no association between total fat or SFA on overall CVD events or death but there was an inverse association between SFA intake and stroke incidence. Again, in the supplemental materials, details of the top five

contributors in each of the countries included in the study to total fat and SFA intake were provided but these were not accumulative by food group.

The SUN (Seguimiento Universidad de Navarra) PCS has been recruiting and collecting data of recent graduates from Navarra University on a continuous and dynamic basis since 1999. Included in the analysis of the SUN study presented by Dominguez et al. (2018) were data from 4,878 participants aged ≥45 years with a mean follow-up of 9.5 years. The aim was to establish whether it was SFA or total fat, red or processed meat (as a source of SFA) that was associated with mortality, including CVD mortality in the cohort. Over the follow-up period 45 deaths from CVD occurred. After multi-variate adjustments (including energy intake) there was no evidence that one additional serving of red meat (or of total meat for that matter) per day increased the hazard ratio of risk from CVD. The association between red or processed meat or SFA and CVD mortality was not reported. However, there was no significant association between SFA and all-cause mortality. Dietary data was collected at baseline via FFQ, which might have been a confounding factor as dietary habits might have changed over the 9.5 years follow-up period. As the authors highlighted, the cohort was highly educated and presented with a number of lifestyle factors (low BMI, high physical activity levels) that were not representative of the average population. However, this presented the opportunity to assess the association between SFA and a particular source of SFA in the absence or reduction of other significant CVD confounders.

Zhuang et al. (2019) most recently presented findings from the National Institutes of Health - American Association of Retired Persons Diet and Health Study, which included data from 521,120 participants (50 to 71 years) enrolled between 1995 and 1996. Follow-up was 16 years. Cardiovascular mortality was linearly and positively associated with SFA, especially in men; mortality from T2DM was also positively, but not linearly, associated with SFA. Dietary intake was assessed via FFQ at baseline only. Although the study undertook two validation exercises with subsamples of the cohort to estimate correlations between actual and FFQ intake and between nutrient intake and practical intakes, the limitations of the time gap between dietary reporting and follow-up remain. The diet might have changed so that other factors might have been greater contributors to death. Furthermore, the authors did not provide any details regarding the sources of the SFAs, so that no potential differential effects could be examined.

### 1.6 The reviews by the Scientific Advisory Committee on Nutrition on the impact of dietary carbohydrates and saturated fatty acids on individual clinical markers of cardiometabolic risk

The SACN report on CHO (2015) and the SACN draft report on SFA (2018) also examined the impact of CHO and SFA on individual markers of CM risk, namely LDL-C, HDL-C, TG, BP and blood lipid ratios. A total of 26 RCTs contributed to the evidence-base that resulted in the conclusion of adequate evidence of no effect on higher CHO, lower fat diets on LDL-C concentrations. The combined findings from 28 RCTs showed that there was adequate evidence of no effect on higher CHO, lower fat diets on HDL-C concentrations. A total of 30 RCTs contributed to the evidence-base of the impact of higher CHO, lower fat, adequate protein diets on TG concentrations. The evidence was deemed to be moderate and showed a biological effect on TG concentrations with these reducing at a lesser rate as compared with a lower CHO, average or higher fat and higher protein diet. However, the reduction of fat intake might play a confounding role in this. The evidence from 26 RCTs included in the MA was deemed to be adequate to conclude that there were no associations between higher CHO, lower fat diets on neither SBP nor DBP. There was limited evidence that total sugar intake had no effect on BP, HDL-C, LDL-C or TG (based on three RCTs for BP, HDL-C and LDL-C and two RCTs for TG). Four SR (three with MA) contributed to the findings that the evidence from RCTs was adequate to conclude that reduced intake of SFA lowers LDL-C but the evidence from PCS was insufficient. Evidence from two SR of RCTs was adequate to demonstrate that reduced SFA intake lowers HDL-C. Only one SR of RCTs, which included a MA, was identified. The evidence was deemed adequate that reduced SFA intake lowers TG concentrations. Based on one SR and MA of RCTs there was limited evidence that SFA had no effect on BP. The reviews conducted by SACN on CHO (2015) and SFA (2018) have highlighted that a lot of the evidence informing decision on dietary recommendations on intake of these two macronutrients and CM risk is still inconclusive. Further studies are therefore need to contribute to the body of evidence and elucidate the effects of diet and potential mechanisms that cause these. Relevant additional evidence published since the SACN 2015 and 2018 (draft) reports addressing any of the CM surrogate markers above and potential emerging surrogate markers are discussed in chapter 2 and chapter 3 of this PhD thesis.

### 1.7 The impact of substituting saturated fatty acids with carbohydrates or other fats

Whilst (mechanistic) links between individual macronutrients and CM risk have been well established, the complexity of the utilisation and metabolisation of different energy substrates make it harder to determine the optimal ratio of dietary macronutrients. Over the past few years a number of (systematic) reviews and meta-analyses has considered the effects of substituting SFA with USFA or with CHO (Hooper et al., 2015b; Mensink, 2016; Siri-Tarino et al., 2015). The latest review to do so was the comprehensive SACN report on SFA (2018 (see above)). The majority of the evidence obtained from RCTs and PCSs for the effects of substituting SFA with PUFA, or PUFA and MUFA, or CHO on CVD morbidity and mortality and selected CM risk factors was deemed to be limited or insufficient. Furthermore, the evidence from RCTs proved to be stronger than from PCSs.

There was however adequate or moderate evidence to show that using PUFA to replace SFA lowered both CHD events and mortality, whereas substitution of SFA with CHO increased CHD events. There was also adequate evidence that replacing SFA with either PUFA, MUFA or CHO decreased LDL-C and that substituting SFA with CHO furthermore decreased HDL-C. The evidence that SFA substitution by PUFA decreased fasting glucose concentrations and IR was also deemed to be adequate. Substituting SFA with MUFA also decreased fasting glucose levels but at the same time increased IR. Replacing SFA with CHO increased fasting insulin concentrations, and the evidence for this was deemed to be moderate. No effects on anthropometric markers of adiposity were found when SFA was replaced by either PUFA nor MUFA. The evidence for this was deemed to be adequate.

These findings have important implications for the recommendations made to the general population to prevent and ameliorate CM risk. Whilst the findings were generally not strong, those for one of the currently most important markers of CM risk, LDL-C, showed that substituting SFA with other macronutrients would lower CM risk in light of the mechanistic links between SFA and LDL. Therefore the SACN review would confirm that SFA intake is potentially detrimental to CM health.

However, these substitutions were not necessarily undertaken within the context of a LCHF diet and it can be assumed that the majority of energy obtained from macronutrients would have still been derived from CHO metabolism. Any fats in this context might been part of the diet potentially under energy balance or hypercaloric conditions. This means that there was the potential of these remaining within the circulation for prolonged periods of time exerting atherogenic effects.

Furthermore, the results from the SACN review on the effects of SFA substitution with PUFA, MUFA or CHO showed that more high-quality studies are needed to provide a stronger evidence base for future recommendations.

### 1.8 The ongoing debate surrounding the evidence-base of current dietary guidelines in the context of carbohydrate and fatty acids recommendations

Cardiometabolic diseases are influenced by a number of modifiable factors, including dietary intake (Dehghan et al., 2017; GBD 2017 Diet Collaborators; Grasgruber et al., 2016; Micha et al., 2017; Schwingshackl et al. 2018a; Schwingshackl et al., 2018b). It is for this reason that public health authorities consider the effects of different nutrients on cardiometabolic (CM) risk and prevention (amongst other NCDs) when drafting national nutrition policies and dietary recommendations/guidelines (Jukola, 2019; Levy, 2013; Levy and Tedstone, 2017; Mozaffarian, 2016). In the UK, dietary guidelines are based on recommendations made by the Scientific Advisory Committee on Nutrition (SACN), which reviews the evidence-base for nutrients and foods. The most recent report on carbohydrates (CHO) (SACN, 2015) informed the current dietary recommendations, the Eatwell Guide (EWG), which was published in March 2016 (PHE, 2016a; PHE, 2016b, PHE, 2016c). A review on saturated fatty acids (SFA) (SACN, 2018), which was recently undertaken, should inform future dietary guidance in the UK. Compared to the previous recommendations the amount of CHO to be consumed increased from 47% total energy (%TE) to 50%TE (SACN, 2015), whilst the recommendations for the intake of total fats as ≤33%TE and of SFA in particular as ≤10%TE have remained unchanged since 1991 (COMA, 1991; COMA, 1994, SACN, 2018). This stance is in accordance with guidance issued by other nations (Buyken et al., 2018; Eilander et al., 2015).

The recommendations for CHO, total fat and SFA remain at the heart of ongoing and at times heated discussions with questions continued to be raised regarding their validity (DiNicolantonio et al., 2016; Grasgruber et al., 2016; Harcombe, 2017a; Hu and Bazzano, 2014). Although not necessarily in opposition to them, Sanders (2016) stated there was no evidence for the current lower limits for CHO and upper limits for total fats in the UK dietary guidelines. Nonetheless, Mann et al. (2016) have argued that, as long as there is insufficient evidence on which types of SFA and SFA-rich foods might be beneficial or detrimental to health, the advice to reduce SFA intake should remain. Further evidence is needed to design future dietary recommendations with more differential perspectives on SFA and their sources (Panth et al., 2018).

Critics of the lower limit for CHO intake argue that CHO-rich foods are actually detrimental for human health and state that the guidelines also do not differentiate sufficiently between

quality of CHOs and their food sources. This meant that potential negative effects of refined CHOs were disregarded (DiNicolantonio et al., 2016). Others present evidence that lower CHO intake might be more beneficial for health (Deahghan et al., 2017; Hu and Bazzano, 2014; Gjuladin-Hellon et al., 2019).

Those opposing the limits set for total fat and SFA argue that there is either not sufficient evidence to impose those limits or that evidence to the contrary (fat is beneficial and SFA does not exert harmful effects) is ignored (Nissen, 2016). Hoenselaar (2012) re-examined the evidence-base for SFA used by three different committees (USA and EU-based) to prepare dietary guidelines. Not only were there discrepancies in the interpretation of the original studies by the committees but additional studies were also identified, which would have been available to the committees at the time but were not considered. Furthermore, Hoenselaar (2012) strongly criticised that only one aspect of CM risk (LDL-C) was used in the decision-making process while other factors, which might be equal or better indicators of CVD morbidity and mortality (e.g. HDL-C and the TC/HDL-C ratio) were disregarded. Overall, the author came to the conclusion that "results and conclusions about saturated fat intake in relation to CVD, from leading advisory committees, do not reflect the available scientific literature" (p.122). Other authors have since concluded that there was and still is insufficient evidence to limit SFA intake as a strategy of lowering mortality from specific CMDs (Harcombe et al., 2016; Harcombe et al., 2017a; Harcombe et al., 2017b). Griffin (2015) has challenged this and argued that these critiques based their arguments on limited evidence (small number of studies with small cohorts and trial duration that was too short to show long-term effects). They also neglected to acknowledge that different subgroups of fatty acids exert different effects on surrogate marker of CM risk. This was reflected upon in more detail throughout this thesis.

### 1.9 Aims and objectives of the present PhD research

The aim of this PhD research was to assess the potential impact of dietary macronutrient intake on CM risk factors with focus on CHO and fats. Due to the ongoing and at times heated debate surrounding the issues of total CHO intake and the intake of total fats and SFA in particular these became the focus of the research.

The research was undertaken in two stages. The first study made use of an under-utilised tool enabling thorough glances into the UK population's dietary intake and CM health status, the National Diet and Nutrition Survey Rolling Programme (NDNS RP) 2008/09-2013/14 dataset (chapter 2). This important survey collects a wide range of food intake, nutrition, lifestyle and health-related data that can provide vital insights into the impact of CHO and fats and CM risk factors. Whilst the candidate recognises that there is an outstanding

question regarding the effectiveness (and indeed necessity) of replacing SFA with unsaturated fatty acids (USFA), these were not addressed in the present research study. Focus was on the NDNS as a cross-sectional study capturing dietary intake (within the limitations of the dietary assessment methods used). Whilst other studies have applied simulation modelling to assess the potential impact of isocaloric replacements of one dietary macronutrient for another (Dehghan et al., 2017; Dominguez et al., 2018; Seidelman et al., 2018), this was not the remit of the present research.

The findings from the first study informed the design of the second stage of the PhD (chapter 3). This took the shape of a randomised pilot/feasibility study examining the impact of CHO and fats on not only established but also emerging markers of CM risk. Participants were randomised to either follow a low-carbohydrate, high-fat diet (LCHF) or adhere to the UK dietary guidelines for eight weeks. Assessment of anthropometric and blood plasma markers of CM risk was undertaken at baseline, interim point and endpoint. In addition, data of physical activity were collected through an objective measure.

By analysing a nationally representative data set for aspects of CMD not investigated in this form before and by translating the findings to a randomised pilot study this PhD research makes a novel contribution to the growing body of evidence that recommendations on dietary macronutrient intake can no longer be based on reductionist approaches.

# Chapter 2 – Study 1: Dietary intake and cardiometabolic risk in the National Diet and Nutrition Survey Rolling Programme 2008-2014

## 2.1 Introduction

Data sets compiled from national population-wide surveys have become an increasingly useful tool to provide insights into dietary intake, nutritional status, lifestyle and health and disease status of the respective general population. They have been invaluable in monitoring adherence to dietary guidelines and in defining and developing national and international public health promotion policies and practices (Ahluwalia et al., 2016; Bates et al., 2019; Grandjean, 2012; Rippin et al., 2018). In the UK, it is the National Diet and Nutrition Survey (NDNS) for which these data are collected (NatCen Social Research et al., 2017). Initially the survey was undertaken as individual, separate studies but it has been implemented as an ongoing, rolling programme since 2008 (Bates et al., 2014). This cross-sectional survey has been designed to be representative of the UK free-living general population aged ≥1.5 years. The wide range of data collected include household composition, dietary intake, eating habits inside and outside the home, sun exposure, sleep patterns, physical activity, anthropometrics and urine and blood plasma markers of health and disease. The NDNS informs the work of the UK's Scientific Advisory Committee on Nutrition's (SACN) (Bates et al., 2019), has been used to monitor adherence to the UK Governments dietary guidelines and was instrumental in devising the current dietary recommendations (*The Eatwell Guide* (Scarborough et al., 2016)).

The NDNS data sets are publicly accessible (on application) from the UK Data Service and have been analysed for a variety of purposes by food and nutrition researchers and academics. For the adult population this included: food consumption inside and outside of the home, and dietary quality (Adams et al., 2015; Adams and White, 2015, Fitt et al., 2013; Gaal et al., 2018; Gibson et al., 2016; Goffe et al., 2017; Murakami and Livingstone, 2016; Mann et al., 2015; Mann et al., 2017; Noorwali et al., 2018; Rauber et al., 2018), macronutrient intake (Guess, 2017; Hutchinson et al., 2018; Wang et al., 2019), micronutrient intake (Derbyshire, 2017; Derbyshire, 2018; Ziauddeen et al., 2018) and adherence to dietary guidelines (Giabbanelli and Adams, 2016; Jones et al., 2018; Rosso and Giabbanelli, 2018; Yau et al., 2018).

Surprisingly, only a very limited number of studies to date have undertaken secondary analysis of the NDNS data set to investigate the impact of diet on surrogate endpoints of CMD. Most recently, Patel et al. (2018) compared the consumption of low-calorie beverages and sugar-sweetened beverages, overall diet quality and CM risk factors in UK adults aged

16 and over and found no statistically significant differences in HDL-C, LDL-C, TG and GLUC concentrations between the groups. McGeoghegan et al. (2015) used the data to analyse the association between dietary patterns and the risk of T2DM and found that an anti-oxidant and anti-inflammatory profile (high in fruit and vegetables and low in sugar and white bread) reduced the risk of presenting with the disease. Carroll et al. (2016) undertook a secondary analysis on the NDNS data for those aged ≥16 and ran logistic regression models to investigate the associations between plain water intake and HbA1c status and the odds of presenting with increased CM risk. They found that plain water decreased these odds by 22% in men. The association of whole grain foods with other food groups and markers of CM risk, such as HDL-C, LDL-C, TG and HbA1c was investigated by Mann et al. (2015) by analysing the NDNS data set. There was no association between whole grain consumption and any of these markers. However, the researchers pointed out that this might have been because over 70% of the sample did not consume the minimum recommended amounts making it difficult to detect any statistically significant differences.

Other countries have been far more active in realising the potential of data mining their national nutrition surveys within the context of diet and cardiometabolic risk, such as the Korean National Health and Nutrition Examination Survey Rolling Programme (KNHANES). The data collected through this national survey have been analysed a number of times over the past decade to investigate potential associations between diet and cardiometabolic risk including: macronutrient intake (Ha et al., 2018a; Ha et al., 2018b; Song and Song, 2018), micronutrient intake (Choi and Bae, 2013; Shin et al., 2013) and intake of specific foods or food groups (Kim and Je, 2018; Kwon et al., 2010; Lee and Cho, 2017). Some work has also been undertaken to investigate dietary intake and CM risk in the US population using the National Health and Nutrition Examination Survey Rolling Programme (NHANES). As the majority of the total NHANES sample is white Caucasian as in the NDNS, some of the NHANES studies were discussed in more detail in chapter 2.4.

**Study 1 Aims, objectives and hypothesis**

There has been a scarcity of research examining the impact of dietary intake on surrogate markers of CMD risk in the UK population that makes use of the data available through the NDNS RP. The aim of the present study was therefore to investigate links between macronutrient intake (CHO and fats) and the risk of presenting with MetS.

The objectives were to

- Undertake a secondary analysis of NDNS RP data collected in survey years 2008 – 2014 stratified by sex to investigate whether there were significant differences for demographics, lifestyle factors, CM risk factors and dietary intake
- Undertake a secondary analysis of NDNS RP data collected in survey years 2008 – 2014 stratified by age group for demographics, lifestyle factors, CM risk factors and dietary intake
- Undertake a secondary analysis of NDNS RP data collected in survey years 2008 – 2014 stratified by body mass index category for demographics, lifestyle factors, CM risk factors and dietary intake
- Undertake a secondary analysis of NDNS RP data collected in survey years 2008 – 2014 stratified by sex and grouped into quartiles of dietary intake of total CHO, total starch, total sugars, total fats and SFA to investigate whether there were differences in CM risk between groups
- Undertake a secondary analysis of NDNS RP data collected in survey years 2008 – 2014 stratified by age group and grouped into quartiles of dietary intake of total CHO, total starch, total sugars, total fats and SFA to investigate whether there were differences in CM risk between groups
- Undertake a secondary analysis of NDNS RP data collected in survey years 2008 – 2014 stratified by body mass index category and grouped into quartiles of dietary intake of total CHO, total starch, total sugars, total fats and SFA to investigate whether there were differences in CM risk between groups

Based on the available evidence presented in chapter 1 the candidate hypothesised that those consuming higher amounts of SFA would be at higher CM risk, whereas there would be no association between CM risk and CHO intake.

## 2.2 Methods

### 2.2.1 Sampling and recruitment in the National Diet and Nutrition Survey Rolling Programme

To ensure that the NDNS is representative of the free-living UK population aged 1.5 years and above a multi-stage stratified random sampling approach is applied. The full sampling method has been described in detail elsewhere (Bates et al., 2017). To summarise, the NDNS sample was drawn from the Postcode Address File's (PAF) 'small users' sub-file. The 'small users' sub-file contains a list of all addresses receiving fewer than 25 articles of mail per day (Bates et al., 2014b). These were clustered into Primary Sampling Units (PSUs), which are postcode sector-based small geographical areas, and which are randomly selected from

across the UK. From these PSU clusters a list of addresses (27 or 28 depending on survey year) were selected at random, and from these addresses individual households (catering units) consisting of up to one adult and up to one child selected for the core sample. Boost samples from additional addresses in Northern Ireland, Scotland and Wales were selected at random in the devolved countries to ensure comparability between the four UK nations. This method was deemed the most cost-effective.

Information about the survey was posted to each selected address. This was followed up by a visit from an allocated stage 1 interviewer to each address. The purpose of this visit was to establish whether the address was a residential dwelling and whether it consisted of only one or multiple dwellings. At addresses with more than one residential dwelling the interviewer selected one dwelling unit and from this one catering unit (where there were two or more per dwelling) at random using a Kish grid. This framework reduces interviewer bias. Across the top of the grid the number of units within the sampling area is listed and each is being assigned a random number. It is this number that determines which sampling unit is to be selected (Bates et al., 2014). From each selected catering unit (household) the interviewer then randomly selected up to one adult and up to one child as survey participants. The annual goal was to collect core sample data from 500 adults (aged 19 and over) and 500 children (aged 1.5 to 18 years).

### 2.2.2 Data collection and tools used

Details of tools used for data collection in the NDNS are available elsewhere (Bates et al., 2014). Briefly, a four-day food diary (FD) was used to collect data on dietary intake, the Recent Physical Activity Questionnaire (RPAQ) (MRC, undated; accessible at http://www.mrc-epid.cam.ac.uk/wp-content/uploads/2014/08/RPAQ.pdf (Appendix A)) was used to measure habitual physical activity across four areas (home, job, commute and leisure). Height, weight, waist circumference and hip circumference were measured using a standard protocol. A fasted blood sample was drawn to assess markers of nutritional intake, health and disease status. Data collection took place in two stages. During Stage 1 a researcher administered the FD and the RPAQ and measured participants' height and weight. Stage 2 was nurse-led with further anthropometric measurements taken and a fasted blood sample collected from those participants that consented to it.

### 2.2.3 Weighting and effect size

As it was possible at each stage of the selecting process for selection bias to occur, either through the difference in sample selection (unequal selection probability) or in participant response (through participant drop-out or non-response) the sample was weighted

throughout the different stages. Final weights were created to combine the selection weights and the non-response weights. These are described elsewhere (Bates et al., 2016). Briefly, weights were created to allow for analysis of data that only included household level, dietary intake, smoking and drinking data. A second weight was created for data collected during the nurses visit, such as further anthropometrics and blood pressure data. A third weight is to be used for data obtained from the analysis of the blood samples. Another weight is for the analysis of RPAQ data only. There are further weights, but these are not of interest for the present analysis. Where an analysis combines data from different collection types, the weight for the chronologically last stage is to be used. For example, for dietary intake data (e.g. CHO consumption) combined with blood sample data (e.g. total cholesterol) the weight for the blood samples has to be applied (NatCen Social Research, Undated).

The cluster design of the NDNS means that the level of precision can be affected (and hence the standard error increase) as individuals within the same cluster tend to be more similar. As a result, the representativeness of the results can be affected. Briefly, this means that whilst the unweighted adult sample consisted of 3,450 adults in Years 1 – 4 (both core and country boost samples combined), the effective adult sample size in Years 1 – 4 was 1,909 with a sample efficiency of 55%. The effective sample size of the unweighted adult sample giving a blood sample (n=1,769) was n=908 (51%) in Years 1 – 4 (Tipping, 2014). Figures for the effective sample sizes for Years 5 and 6 have not been reported yet.

### 2.2.4 Definition of the metabolic syndrome

Metabolic Syndrome was defined according to the criteria set by the International Diabetes Federation (IDF) (Alberti et al., 2009). Participants were classified as having metabolic syndrome if they presented with three out of five factors displayed in Table 2.1.

Table 2.1 – Characteristics of the metabolic syndrome as defined by IDF

| Measure | Cut-off point |
|---|---|
| Increased waist circumference | ≥94cm in men and ≥80cm in women of white Caucasian ethnicity* |
| Increased triglyceride concentrations (or drug treatment against) | ≥1.7 mmol/L |
| Reduced HDL-C concentrations (or drug treatment against) | ≤1.0 mmol/L in men<br>≤1.3 mmol/L in women |
| Increased systolic (SBP) and or diastolic (DBP) blood pressure (or drug treatment against) | ≥130 mmHg for SBP<br>≥85 mmHg for DBP |
| Increase fasting glucose (or drug treatment against) | ≥5.6 mmol/L |

*based on UK-specific cut-offs (NHS, 2016)

### 2.2.5 Study sample selection

To achieve a more homogenous sample a number of exclusion criteria were applied for the present analysis. Only data from adults (as defined by the NDNS as age ≥19 years) were used, the sample was limited to the population that identified as white. Those with a BMI below 18.5 kg/m$^2$ and above a BMI above 39.9 kg/m$^2$ were excluded. The final unweighted sample for the purpose of the present analysis was n=2217 (M =924, F = 1293).

### 2.2.6 Statistical analysis

Data sets for household level information (including blood sample and anthropometric data) and individual dietary intake were merged as were the data sets for the 2008/09 – 2011/12 and 2013/14 survey waves. The data from the combined data set was reweighted using the approach suggested by O'Muircheartaigh and Pedlow (2002) (cited in Bates et al., 2016) to account for the complex sample design of the NDNS. The appropriate weights for each collection stage were applied in the analysis following NDNS guidance as described above (Chapter 2.2.3).

Due to the design of the NDNS RP survey all data for the present study were analysed using the complex survey function, which is the recommended and appropriate methods for nationally representative data sets. The final sample was drawn from a stratified and clustered population rather than from a simple random sample. These factors are taken into account in the complex survey mode of statistical packages to address issues such as potential over- or underestimation of variability, precision and standard errors (Lumley, 2004).

Separate unadjusted general linear models were built to assess differences in mean estimates [95% confidence interval (CI)] for general characteristics of the total sample, and between men and women, between BMI groups (18.5-24.9 kg/m$^2$, 25.0-29.9 kg/m$^2$ and 30.0-39.9 kg/m$^2$) and between adult age groups (19-34, 35-49, 50-64 and ≥65 years). These were calculated for age (not for the age groups), BMI (not for the BMI groups), moderate-to-vigorous physical activity (MVPA), smoking status and prevalence of metabolic syndrome using IBM SPSS Statistics for Windows, Version 25.0 (Armonk, NY: IBM Corp. Released 2017). Separate unadjusted general linear models were built to assess differences in mean estimates [95% confidence interval (CI)] between genders (M/F), BMI groups (18.5-24.9kg/m$^2$, 25.0-29.9kg/m$^2$ and 30.0-39.9kg/m$^2$) and adult age groups (19-34, 35-49, 50-64 and ≥65 years) for HDL-C, LDL-C, TG, GLUC, WC, DBP, SBP, and TG/HDL-C ratio (with the appropriate weighting factor (see above)).

Separate unadjusted general linear models were then built to assess differences in mean estimates [95%CI] between those with and without metabolic syndrome in the total population, men, women, BMI groups 18.5-24.9kg/m$^2$, 25.0-29.9kg/m$^2$ and 30.0-39.9kg/m$^2$ and adult age groups 19-34, 35-49, 50-64 and ≥65 years for HDL-C, LDL-C, TG, GLUC, WC, DBP, SBP, and TG/HDL ratio (with the appropriate weighting factor (see above)).

Separate unadjusted and adjusted general linear models were then built to assess differences in mean estimates [95%CI] of dietary intake of total energy, total carbohydrates (CHO), total starch (STAR), total sugars (SUG), total fat (FAT) and saturated fatty acids (SFA) between those with and without MetS in the total population, men, women, BMI groups 18.5-24.9kg/m$^2$, 25.0-29.9kg/m$^2$ and 30.0-39.9kg/m$^2$ and adult age groups 19-34, 35-49, 50-64 and ≥65 years for HDL-C, LDL-C, TG, GLUC, WC, DBP, SBP, and TG/HDL-C ratio (with the appropriate weighting factor (see chapter 2.2.3)) .

The models for total energy intake for the total population were adjusted for sex, age, BMI, number of cigarettes smoked per day and daily moderate to vigorous physical activity. The models for total energy intake for men and women were adjusted for age, BMI, number of cigarettes smoked per day and daily moderate to vigorous physical activity. The models for total energy intake for BMI groups were adjusted for sex, age, number of cigarettes smoked per day and daily moderate to vigorous physical activity. The models for total energy intake for the age groups were adjusted for sex, BMI, number of cigarettes smoked per day and daily moderate to vigorous physical activity.

The models for macronutrient intake for the total population were adjusted for gender, age, BMI, number of cigarettes smoked per day, daily moderate to vigorous physical activity and total energy intake. The models for macronutrient intake for men and women were adjusted for age, BMI, number of cigarettes smoked per day, daily moderate to vigorous physical activity and total energy intake. The models for macronutrient intake for BMI groups were adjusted for age, gender, number of cigarettes smoked per day, daily moderate to vigorous physical activity and total energy intake. The models for macronutrient intake for age groups were adjusted for BMI, gender, number of cigarettes smoked per day, daily moderate to vigorous physical activity and total energy intake.

For all general linear models an $\alpha < 0.05$ was used to determine statistical significance.

For the total population, men, women, BMI groups 18.5-24.9kg/m$^2$, 25.0-29.9kg/m$^2$ and 30.0-39.9kg/m$^2$ and adult age groups 19-34, 35-49, 50-64 and ≥65 years separate quartiles

were created for intake (as percentage total energy (%TE)) of CHO, STAR, SUG, FAT and SFA.

Using logistic regression analysis separate unadjusted and adjusted models were run to assess the odds ratio (OR) [95% CI] and adjusted odds ratio (AOR) [95% CI] of presenting with MetS dependent on intake (as percentage total energy (%TE)) CHO, STAR, SUG, FAT and SFA. The highest quartile was used as reference category. The adjustments for each separate group were undertaken as described above.

Although the p-value will be reported for the logistic regression, analyses focused on interpretation of the 95%CI to account for the fact that statistical significance does not equal clinical significance and does not address effect size (Coulson et al., 2010; Ferrill et al., 2010; Nakagawa and Cuthill, 2007; Wasserstein and Lazar, 2016). In line with common practice results were regarded as significant if the CI did not include the null value, which was in this case OR=1.0 or AOR=1.0 (Attia, 2005; Szumilas, 2010). Whilst the odds ratios addressed the effect of dietary intake (Nakagawa and Cuthill, 2007), the CI permitted to assess the reliability and the precision of the results obtained (Coulson et al., 2010; Ferrill et al., 2010; Nakagawa and Cuthill, 2007) and the strength of the effect (Du Prel et al., 2009). A large CI indicated a low level of precision and a small CI a high level of precision.

Descriptive analysis was performed using IBM SPSS Statistics for Windows, Version 24.0 (Armonk, NY: IBM Corp. Released 2016). General linear models and logistic regression analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (Armonk, NY: IBM Corp. Released 2017).

### 2.2.7 Ethical approval

Ethics approval for the NDNS RP 2008/09 – 2012/13 (survey years 1 – 5) was obtained from the Oxfordshire A Research Ethics Committee (Ref. No. 07/H0604/113) (Bates et al., 2014; Bates et al., 2016). Ethic approval for the NDNS RP 2013/14 (survey year 6) was obtained from the Cambridge South NRES Committee (Ref. no. 13/EE/0016) (Bates et al., 2016). All letters of approval for both the original ethics application and any substantial amendments granted afterwards plus approved documents were forwarded to all Local Research Ethics Committees (LREC) for areas in which fieldwork was being conducted. Where required an application for research governance's approval to the appropriate Research and Development Committees for all participating NHS laboratories was made and approval obtained (Bates et al., 2014; Bates et al., 2016). As the present analysis uses secondary data no ethical approval from Liverpool John Moores University's Research Ethics Committee was required.

## 2.3 Results

### 2.3.1 General characteristics of population

Details of the general characteristics are presented in Table 2.2. The unweighted total sample consisted of 2217 adults aged ≥19. In the weighted sample 51.7% of participants were female. Woman were on average non-significantly older than men ($p$=.112) and presented with a non-significantly lower BMI ($p$=.087). They spent significantly less time in moderate to vigorous physical activity ($p$<.0001). Significantly more men than women were classed as current smokers ($p$=.011). Over 32% of the total population presented with MetS but significantly more men than women presented with MetS ($p$<.0001). The 19-34 age group presented with the lowest BMI and the 50-64 age group presented with the highest BMI. The difference were only significant between the youngest and the oldest age group ($p$<.0001). The first three age groups spent similar amounts of time at moderate-to-vigorous physical activity (MVPA) but the ≥65 age group did so significantly less, about half that of the other age group (all $p$<.0001). The youngest age group had the highest percentage of current smokers (24.8%) and the oldest age group the lowest percentage (7.5%) ($p$<.0001). Prevalence of MetS increased significantly with each age group and was nearly 300% times higher in the 35-49 age group compared to the 19-34 age group, and even higher in the older age groups (all $p$<.0001). The overweight population was the oldest of the sample. Physical activity levels were similar between BMI groups. The normal-weight population had the highest percentage of smokers (22.7%) and significantly more so than the obese popuulation ($p$=.020). Prevalence of MetS was positively and significantly associated with BMI group with 57.2% of those classed as obese presenting with MetS ($p$<.0001).

Table 2.2 – General characteristics of sample

| | | Weighted % | Age (years) | p | BMI (kg/m$^2$) | p | MVPA (hrs/d) | p | Current smoker (%) | p | MetS (%) | p | UWC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Total** | | - | 49.4 [48.5,50.4] | - | 27.1 [26.9,27.4] | - | 1.72 [1.54,1.90] | - | 17.8 | - | 32.5 | - | 2217 |
| **Sex** | **M** | 48.3 | 48.6 [47.3,49.9] | .112 | 27.3 [27.0,27.8] | .087 | 2.4 [2.1,2.7] | **<.0001** | 20.2 | **.011** | 38.4 | **<.0001** | 924 |
| | **F** | 51.7 | 50.2 [48.8,51.5] | | 26.9 [26.5,27.3] | | 1.1 [0.9,1.2] | | 15.6 | | 26.4 | | 1293 |
| **Age group** | **19-34** | 22.5 | - | - | 25.3 [24.8,25.8] | **<.0001** | 2.0 [1.6,2.3] | **<.0001** | 24.8 | **<.0001** | 8.1 | **<.0001** | 421 |
| | **35-49** | 28.7 | - | | 27.5 [27.0,28.1] | .853 | 2.0 [1.6,2.3] | **<.0001** | 24.7 | **<.0005** | 23.7 | **<.0001** | 697 |
| | **50-64** | 27.8 | - | | 27.9 [27.4,28.3] | .266 | 1.8 [1.5,2.0] | **<.0001** | 16 | **.019** | 42.7 | **<.0001** | 631 |
| | **≥65** | 21.2 | - | | 27.5 [27.0,28.0] | Reference category | 1.0 [0.8,1.2] | Reference category | 7.5 | Reference category | 60.7 | Reference category | 468 |
| **BMI group** | **18.5-24.9** | 36.3 | 44.9 [43.4,46.3] | **<.0001** | - | - | 1.8 [1.5,2.1] | .531 | 22.7 | **.020** | 11.7 | **<.0001** | 766 |
| | **25-29.9** | 37.6 | 52.1 [50.7,53.6] | .737 | - | | 1.7 [1.4,1.9] | .939 | 14.9 | .879 | 38.7 | **<.0001** | 847 |
| | **30-39.9** | 26.0 | 51.7 [49.9,53.6] | Reference category | - | | 1.7 [1.3,2.0] | Reference category | 15.2 | Reference category | 57.2 | Reference category | 604 |

BMI – body mass index; f – female; m – male; MetS – metabolic syndrome; MVPA – moderate to vigorous physical activity; UWC – unweighted count; data are presented as mean [95% confidence interval]

### 2.3.2 Cardiometabolic risk markers in the UK general population

There were significant differences between men and women for a number of cardiometabolic risk markers, which are detailed in Table 2.3a, 2.3b and 2.3c. Women presented with significantly higher HDL-C concentrations ($p<.001$) and significantly lower TG ($p<.001$) and GLUC concentrations ($p<.001$). They also had significantly smaller WC ($p<.024$) and significantly lower SBP ($p<.001$). Compared to women men presented with a significantly higher TG/HDL ratio ($p<.001$). There were significant differences between age groups for LDL-C, TG, GLUC (all $p<.001$), WC ($p<.001$), DBP ($p<.001$) and SBP ($p<.001$). The highest mean estimate concentrations of LDL-C, TG, and DBP occurred in the 50 – 64 age group. Concentrations/values of GLUC, WC and SBP significantly and consistently increased across age groups with the ≥65 age group presenting with the highest GLUC concentrations, the highest WC and the highest SBP. There were significant differences between BMI groups for HDL-C, LDL-C, TG, GLUC, WC, DBP, SBP, and TG/HDL (all $p<.001$). All levels were positively associated with increasing BMI group with the exception of HDL-C, which showed an inverse association.

Table 2.3a – Cardiometabolic risk markers of white UK adult population by sex

| | **Sex** | | |
|---|---|---|---|
| | **M (n=924)** | **F (n=1293)** | **P** |
| **HDL-C (mmol/L)** | 1.3 [1.3,1.4] | 1.6 [1.6, 1.7] | **<0.001** |
| **LDL-C (mmol/L)** | 3.1 [3.0, 3.2] | 3.2 [3.1, 3.3 | .092 |
| **TG (mmol/L)** | 1.5 [1.4, 1.6] | 1.2 [1.1, 1.2] | **<0.001** |
| **GLUC (mmol/L)** | 5.5 [5.3, 5.6] | 5.1 [5.0, 5.1] | **<0.001** |
| **WC (cm)** | 97.4 [96.4, 98.3] | 87.0 [86.2, 87.9] | **.024** |
| **DBP (mmHg)** | 75 [74, 76] | 73 [72, 74] | .393 |
| **SBP (mmHg)** | 131 [129,132] | 125 [123,126] | **<.001** |
| **TG/HDL** | 1.3 [1.2, 1.4] | 0.8 [0.8,0.8] | **<.001** |

DBP-diastolic blood pressure; F – female; GLUC – Glucose; HDL - High Density Lipoprotein cholesterol; LDL - Low Density Lipoprotein cholesterol; M – male; SBP – systolic blood pressure; TG – triglycerides; WC – waist circumference; data presented as mean [95% confidence interval]

Table 2.3b – Cardiometabolic risk markers of white UK adult population by age group

| | Age group (years) | | | | |
|---|---|---|---|---|---|
| | **19-34 (n=421)** | **35-49 (n=697)** | **50-64 (n=631)** | **≥65 (n=468)** | **P** |
| **HDL-C (mmol/L)** | 1.5 [1.4, 1.5] | 1.5 [1.4, 1.5] | 1.5 [1.5, 1.6] | 1.5 [1.4, 1.5] | 0.97 |
| **LDL-C (mmol/L)** | 2.7 [2.6, 2.8] | 3.3 [3.2, 3.3] | 3.5 [3.4, 3.6] | 3.1 [3.0, 3.3] | **<.001** |
| **TG (mmol/L)** | 1.1 [1.0, 1.3] | 1.4 [1.3, 1.5] | 1.5 [1.4, 1.6] | 1.3 [1.2, 1.4] | **<.001** |
| **GLUC (mmol/L)** | 4.9 [4.8, 5.1] | 5.2 [5.1, 5.3] | 5.4 [5.3, 5.5] | 5.6 [5.4, 5.8] | **<.001** |
| **WC (cm)** | 85.5 [84.2,86.8] | 92.1 [90.8,93.5] | 95.0 [93.7,96.3] | 95.7 [94.2,97.2] | **<.001** |
| **DBP (mmHg)** | 70 [68,71] | 76 [75,78] | 77 [75,78] | 74 [73,75] | **<0.001** |
| **SBP (mmHg)** | 119 [117,120] | 124 [123,126] | 130 [129,132] | 138 [136,141] | **<0.001** |
| **TG/HDL** | 0.9 [0.8,1.0] | 1.1 [1.0,1.3] | 1.2 [1.2,1.3] | 1.0 [0.9,1.1] | 0.064 |

DBP-diastolic blood pressure; GLUC – Glucose; HDL - High Density Lipoprotein cholesterol; LDL - Low Density Lipoprotein cholesterol; SBP – systolic blood pressure; TG – triglycerides; WC – waist circumference; data presented as mean [95% confidence interval]

Table 2.3c – Cardiometabolic risk markers of white UK adult population by BMI group

| | BMI group (kg/m$^2$) | | | |
|---|---|---|---|---|
| | **18.5 – 24.9 (n=766)** | **25-29.9 (n=847)** | **30.0-39.9 (n=604)** | **p** |
| **HDL-C (mmol/L)** | 1.6 [1.6, 1.7] | 1.5 [1.4, 1.5] | 1.3 [1.3, 1.4] | **<.001** |
| **LDL-C (mmol/L)** | 2.9 [2.8, 3.0] | 3.3 [3.2, 3.4] | 3.3 [3.2, 3.5] | **<.001** |
| **TG (mmol/L)** | 1.0 [1.0, 1.1] | 1.4 [1.4, 1.5] | 1.6 [1.5, 1.7] | **<.001** |
| **GLUC (mmol/L)** | 5.0 [4.9, 5.0] | 5.3 [5.2, 5.3] | 5.7 [5.5, 5.9] | **<0.001** |
| **WC (cm)** | 80.7 [80.0,81.4] | 93.6 [92.8,94.3] | 106.0 [104.8,107.3] | **<.001** |
| **DBP (mmHg)** | 71 [70,72] | 75 [74,76] | 78 [77,79] | **<.001** |
| **SBP (mmHg)** | 123 [121,125] | 129 [128,131] | 132 [131,134] | **<.001** |
| **TG/HDL** | 0.7 [0.7,0.8] | 1.2 [1.1, 1.3] | 1.4 [1.2,1.6] | **<.001** |

BMI – body mass index; DBP-diastolic blood pressure; GLUC – Glucose; HDL - High Density Lipoprotein cholesterol; LDL - Low Density Lipoprotein cholesterol; M – male; SBP – systolic blood pressure; TG – triglycerides; WC – waist circumference; data presented as mean [95% confidence interval)

### 2.3.3 Cardiometabolic risk in the UK population with and without metabolic syndrome

Details of CM risk in the total UK population and when stratified by gender can be found in Table 2.4. Those that presented with MetS were significantly older ($p$<.001) than those who did not and fell into the upper end of the overweight category. The mean estimate BMI for the healthy total, male and female population fell just into the overweight category. There were also significant differences in smoking status between groups. As expected with the diagnosis of MetS those presenting with it had significant higher values for all markers contributing to the definition of MetS. This was the case for all strata. Low-density lipoprotein cholesterol concentrations were significantly higher in the healthy group compared to the MetS group ($p$<.001 for the total population and men: $p$<.008 for women). The TG/HDL-C ratio was also consistently and significantly higher in the unhealthy group compared to the healthy group (all $p$<.001).

In all BMI groups those presenting with MetS were significantly older than the healthy groups (all p<.001). However, the age difference was greater in the normal-weight BMI

group. Low-density lipoprotein cholesterol concentrations were significantly lower in the MetS group compared to the healthy group for the overweight category ($p$<.001) and the obese category ($p$=.011). Only in the normal-weight category the MetS group presented with higher LDL-C concentrations than the healthy population ($p$=.005) (Table 2.5).

The data for the four adult age groups can be found in Table 2.6 (19-34 and 35-49 age groups) and Table 2.7 (50-64 and ≥65 age groups). In all age categories the population presenting with MetS had a significantly higher BMI than the healthy population ($p$<.001 for the 19-34, 35-49 and 50-64 age group, $p$=.007 for the ≥65 category). The percentage of smokers was significantly higher in the unhealthy population in the 35-49 age range ($p$=.041) and the unhealthy population in the 50-64 age range ($p$<.001). Differences in LDL-C concentrations were only significant in the 50-64 and ≥65 age groups (all $p$<.001) but concentrations were higher in the healthy group for both age groups.

Table 2.4 – Cardiometabolic risk markers in the total, male and female population with and without metabolic syndrome

| MetS | Total | | | M | | | F | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Y (n=674)** | **N (n=1543)** | **P** | **Y (n=354)** | **N (n=570)** | **P** | **Y (n=320)** | **N (n=973)** | **p** |
| **Age (years)** | 59.0 [57.4,60.5] | 43.8 [42.6,44.9] | **<.001** | 56.5 [54.4,58.5] | 42.5 [40.9,44.1] | **<.001** | 62.7 [60.7,64.7] | 44.9 [43.2,46.7] | **<.001** |
| **BMI (kg/m$^2$)** | 29.5 [29.1,29.9] | 25.5 [25.2,25.8] | **<.001** | 29.5 [29.1,29.9] | 25.7 [25.3,26.0] | **<.001** | 29.5 [28.7,30.3] | 25.4 [24.9,25.8] | **<.001** |
| **MVPA (hrs/d)** | 1.7 [1.3,2.0] | 1.9 [1.6,2.1] | 0.434 | 1.7 [1.3,2.0] | 2.6 [2.2,3.1] | .839 | 0.8 [0.7,1.0] | 1.2 [1.1,1.4] | .246 |
| **Smoking (%)** | 19.0 | 18.0 | **<.001** | 20.5 | 21.3 | **<.001** | 16.7 | 15.2 | **.023** |
| **HDL-C (mmol/L)** | 1.25 [1.21,1.29] | 1.62 [1.59,1.65] | **<.001** | 1.15 [1.12,1.19] | 1.44 [1.40,1.49] | **<.001** | 1.40 [1.34,1.46] | 1.77 [1.73,1.80] | **<.001** |
| **LDL-C (mmol/L)** | 3.04 [2.92,3.16] | 3.15 [3.09,3.22] | **<0.001** | 3.03 [2.87,3.19] | 3.11 [3.02,3.19] | **.008** | 3.05 [2.87,3.24] | 3.19 [3.09,3.29] | **<.001** |
| **TG (mmol/L)** | 1.96 [1.84,2.07] | 1.03 [1.00,1.06] | **<.001** | 2.08 [1.91,2.25] | 1.14 [1.08,1.20] | **<.001** | 1.77 [1.65,1.89] | 0.94 [0.90,0.97] | **<.001** |
| **GLUC (mmol/L)** | 5.93 [5.76,6.11] | 4.94 [4.87,5.01] | **<.001** | 6.13 [5.88,6.37] | 5.05 [4.92,5.18] | **<.001** | 5.65 [5.46,5.84] | 4.85 [4.78,4.92] | **<.001** |
| **WC (cm)** | 101.1 [99.9,102.4] | 86.8 [86.0,87.6] | **<.001** | 104.6 [103.3,106.0] | 91.9 [90.8,92.9] | **<.001** | 95.7 [93.9,97.5] | 82.5 [81.4,83.5] | **<.001** |
| **DBP (mmHg)** | 77.69 [76.48,79.00] | 72.38 [71.59,73.18] | **.01** | 79.08 [77.57,80.58] | 72.74 [71.48,74.00] | **.032** | 75.59 [73.80, 77.38] | 72.08 [71.21,72.95] | .117 |
| **SBP (mmHg)** | 136.33 [134.73,137.91] | 123.31 [122.16,124.47] | **.001** | 136.84 [134.94,138.75] | 126.87 [125.16,128.58] | **.006** | 135.54 [133.18,137.90] | 120.30 [118.92,121.68] | **.001** |
| **TG/HDL** | 1.8 [1.64,1.93] | 0.7 [0.7,0.7] | **<.001** | 2.02 [1.8,2.2] | 0.9 [0.8,0.9] | **<.001** | 1.4 [1.3,1.6] | 0.6 [0.5,0.6] | **<.001** |

BMI – body mass index; DBP-diastolic blood pressure; F – female; GLUC – Glucose; HDL - High Density Lipoprotein cholesterol; LDL - Low Density Lipoprotein cholesterol; M – male; MetS - metabolic syndrome; MVPA – moderate to vigorous physical activity; SBP – systolic blood pressure; TG – triglycerides; WC – waist circumference; data presented as mean [95% confidence interval]

Table 2.5 - Cardiometabolic risk markers in the normal weight, overweight and obese population with and without metabolic syndrome

| **BMI (kg/m²)** | **18.5 – 24.9** | | | **25.0 – 29.9** | | | **30.0 – 39.9** | | |
|---|---|---|---|---|---|---|---|---|---|
| **MetS** | **Y (n=76)** | **N (n=690)** | **P** | **Y (n=310)** | **N (n=537)** | **P** | **Y (n=288)** | **N (n=316)** | **p** |
| **Age (years)** | 64.9 [61.1,68.7] | 40.1 [38.6,41.7] | **<.001** | 58.6 [56.2,61.0] | 47.1 [45.2,49.0] | **<.001** | 57.4 [54.9,59.9] | 49.1 [46.2,52.1] | **<.001** |
| **MVPA (hrs/d)** | 2.0 [0.6,3.3] | 1.8 [1.6,2.1] | .368 | 1.7 [1.3,2.1] | 1.8 [1.4,2.2] | .585 | 1.6 [1.0,2.1] | 2.0 [1.2,2.8] | .347 |
| **Smoking (%)** | 26.9 | 22.2 | **.018** | 16.2 | 14.6 | **.034** | 19.3 | 11.5 | **.001** |
| **HDL-C (mmol/L)** | 1.47 [1.36,1.59] | 1.67 [1.62,1.71] | **<.001** | 1.27 [1.22,1.32] | 1.58 [1.53,1.62] | **<.001** | 1.16 [1.11,1.21] | 1.53 [1.47,1.60] | **<.001** |
| **LDL-C (mmol/L)** | 2.99 [2.78,3.20] | 2.88 [2.80,2.95] | **.005** | 2.96 [2.79,3.13] | 3.39 [3.29,3.49] | **<.001** | 3.14 [2.95,3.34] | 3.53 [3.31,3.75] | **.011** |
| **TG (mmol/L)** | 1.54 [1.32,1.76] | 0.96 [0.92,1.00] | **<.001** | 1.95 [1.76,2.15] | 1.12 [1.06,1.19] | **<.001** | 2.11 [1.90,2.31] | 1.04 [0.98,1.11] | **<.001** |
| **GLUC (mmol/L)** | 5.35 [5.10,5.59] | 4.92 [4.78,5.05] | **.012** | 5.73 [5.58,5.89] | 4.94 [4.89,4.99] | **<.001** | 6.36 [6.00,6.70] | 5.03 [4.96,5.10] | **<.001** |
| **WC (cm)** | 87.0 [85.8,88.3] | 79.4 [78.7,80.2] | **<.001** | 97.8 [96.6,99.0] | 91.2 [90.3,92.0] | **<.001** | 109.3 [107.7,111.5] | 102.8 [101.2,104.3] | **<.001** |
| **DBP (mmHg)** | 75.33 [71.59,79.07] | 69.64 [68.72,70.55] | .106 | 77.26 [75.48,79.03] | 73.68 [72.45,74.92] | **.005** | 78.86 [77.25,80.46] | 78.02 [76.03,80.00] | .226 |
| **SBP (mmHg)** | 136.74 [133.46,140.03] | 119.66 [118.45,120.87] | **.001** | 135.09 [133.00,137.18] | 125.50 [123.74,127.27] | **<.001** | 137.46 [134.92,140.01] | 129.80 [126.50,133.09] | .153 |
| **TG/HDL** | 1.2 [1.0,1.4] | 0.6 [0.6,0.7] | **<.001** | 1.8 [1.5,2.0] | 0.8 [0.7,0.9] | **<.001** | 2.0 [1.7,2.3] | 0.7 [0.7,0.8] | **<.001** |

BMI – body mass index; DBP-diastolic blood pressure; GLUC – Glucose; HDL - High Density Lipoprotein cholesterol; LDL - Low Density Lipoprotein cholesterol; MetS - Metabolic syndrome; MVPA – Moderate to vigorous physical activity; SBP – systolic blood pressure; TG – triglycerides; WC – waist circumference; data presented as mean [95% confidence interval]

Table 2.6 - Cardiometabolic risk markers in the population aged 19-34 years and 35-49 years with and without metabolic syndrome

| | 19-34 | | | 35-49 | | |
|---|---|---|---|---|---|---|
| **MetS** | **Y (n=41)** | **N (n=380)** | **P** | **Y (n=146)** | **N (n=551)** | **P** |
| **BMI (kg/m2)** | 30.2 [28.6,31.9] | 23.9 [23.6,24.3] | **<.001** | 30.3 [29.4,31.2] | 26.4 [25.8,26.9] | **<.001** |
| **MVPA (hrs/d)** | 2.9 [1.2,4.7] | 2.0 [1.6,2.4] | .582 | 2.2 [1.1,3.3] | 2.1 [1.6,2.5] | .513 |
| **Smoking (%)** | 39.4 | 24.8 | .58 | 26.5 | 19.3 | **.041** |
| **HDL-C (mmol/L)** | 1.08 [0.96,119] | 1.54 [1.49,1.60] | **.017** | 1.13 [1.07,1.19] | 1.59 [1.55,1.63] | **<.001** |
| **LDL-C (mmol/L)** | 3.24 [2.93,3,54] | 2.62 [2.50,2.73] | .824 | 3.49 [3.31,3.66] | 3.17 [3.08,3.245] | .173 |
| **TG (mmol/L)** | 2.21 [1.93,2.49] | 0.99 [.93,1.04] | **<.001** | 2.52 [2.24,2.79] | 1.06 [0.98,1.12] | **<.001** |
| **GLUC (mmol/L)** | 5.71 [5.30,6.12] | 4.88 [4.69,5.06] | **<.001** | 5.79 [5.53,6.05] | 4.95 [4.83,5.06] | **<.001** |
| **WC (cm)** | 103.1 [98.3,107.4] | 81.8 [80.6,83.0] | **<.001** | 101.9 [99.4,104.4] | 88.60[87.2,90.0] | **.001** |
| **DBP (mmHg)** | 78.42 [75.9,80.9] | 67.8 [66.5,69.1] | **<.001** | 82.3 [79.3,85.2] | 74.2 [73.1,75.4] | **.001** |
| **SBP (mmHg)** | 128.83 [126.2,131.4] | 117.2 [115.7,118.7] | .164 | 133.4 [130.0,137.0] | 121.20 [119.7,122.7] | **<.001** |
| **TG/HDL** | 2.1 [1.9,2.4] | 0.7 [0.6,0.7] | **<0.001** | 2.4 [2.1,2.8] | 0.7 [0.7,0.8] | **<0.001** |

BMI – body mass index; DBP-diastolic blood pressure; GLUC – Glucose; HDL - High Density Lipoprotein cholesterol; LDL - Low Density Lipoprotein cholesterol; MetS - Metabolic syndrome; MVPA – Moderate to vigorous physical activity; SBP – systolic blood pressure; TG – triglycerides; WC – waist circumference

Table 2.7 - Cardiometabolic risk markers in the population aged 50-64 years and ≥65 years with and without metabolic syndrome

| MetS | 50-64 Y (n=241) | 50-64 N (n=390) | p | ≥65 Y (n=246) | ≥65 N (n=222) | P |
|---|---|---|---|---|---|---|
| **BMI (kg/m2)** | 29.9 [29.2,30.6] | 26.2 [25.7,26.6] | **<.001** | 28.6 [27.8,29.3] | 26.2 [25.2,27.1] | **.007** |
| **MVPA (hrs/d)** | 1.9 [1.5,2.3] | 1.9 [1.5,2.3] | .859 | 1.0 [0.8,1.2] | 1.0 [0.7,1.3] | .792 |
| **Smoking (%)** | 21.7 | 12.4 | **<.001** | 9.4 | 6.2 | .053 |
| **HDL-C (mmol/L)** | 1.28 [1.23,1.34] | 1.69 [1.62,1.76] | **<.001** | 1.33 [1.26,1.40] | 1.75 [1.69,1.81] | **<.001** |
| **LDL-C (mmol/L)** | 3.09 [2.90,3.28] | 3.68 [3.55,3.82] | **<.001** | 2.71 [2.51,2.90] | 3.60 [3.42,3.79] | **<.001** |
| **TG (mmol/L)** | 2.09 [1.87,2.31] | 1.05 [0.99,1.12] | **<.001** | 1.46 [1.35,1.56] | 1.03 [0.95,1.10] | **<.001** |
| **GLUC (mmol/L)** | 5.98 [5.80,6.16] | 5.00 [4.95, 5.06] | **<.001** | 6.01 [5.64,6.39] | 5.01 [4.94,5.07] | **<.001** |
| **WC (cm)** | 101.8 [99.9,103.7] | 89.7 [88.1,91.3] | **<.001** | 99.7 [97.4,102.1] | 91.0 [88.7,93.2] | **.003** |
| **DBP (mmHg)** | 79.7 [78.0,81.4] | 75.0 [73.4,76.5] | **.017** | 73.60 [72.0,75.2] | 74.7 [72.8,76.7] | .071 |
| **SBP (mmHg)** | 135.94 [133.6,138.3 ] | 126.56 [124.4,128.8] | **<.001** | 139.12 [136.8,141.6] | 137.79 [135.0,140.6] | .707 |
| **TG/HDL** | 1.9 [1.6,2.2] | 0.7 [0.6,0.8] | **<.001** | 1.2 [1.1,1.4] | 0.6 [0.6,0.7] | **<.001** |

BMI – body mass index; DBP-diastolic blood pressure; GLUC – Glucose; HDL - High Density Lipoprotein cholesterol; LDL - Low Density Lipoprotein cholesterol; MetS - Metabolic syndrome; MVPA – Moderate to vigorous physical activity; SBP – systolic blood pressure; TG – triglycerides; WC – waist circumference

### 2.3.4 Dietary intake and cardiometabolic risk in the UK population with and without metabolic syndrome

#### 2.3.4.1 Dietary intake and cardiometabolic risk in the total, the male and the female population with and without metabolic syndrome

Table 2.8 presents the total energy, CHO, starch, total sugar, total fat and saturated fatty acid intake in the total UK population with and without MetS. There were no significant differences between groups in unadjusted nor adjusted models.

Table 2.8 – Total energy, carbohydrate and fat intake in the total UK white population with and without metabolic syndrome ( adjusted and unadjusted models)

| | | Total | | |
|---|---|---|---|---|
| | | MetS | | |
| | Model | Y (n=674) | N (n=1543) | p |
| **TE** | **1** | 1862.1 [1802.7,1921.6] | 1905.8 [1866.9,1944.8] | .209 |
| | **2** | 1882.4 [1824.8,1940.0] | 1893.9 [1848.3,1939.6] | .712 |
| **CHO** | **1** | 45.5 [44.8,46.2] | 45.9 [45.3,46.5] | .427 |
| | **2** | 46.5 [45.4,47.6] | 45.5 [45.3,46.5] | .247 |
| **STAR** | **1** | 25.8 [25.1,26.4] | 25.8 [25.4,26.2] | .952 |
| | **2** | 26.5 [25.6,27.5] | 25.7 [25.3,26.2] | .200 |
| **SUG** | **1** | 19.8 [19.0,20.5] | 20.1 [19.7,20.6] | .437 |
| | **2** | 20.0 [19.0,20.9] | 19.8 [19.3,20.3] | .885 |
| **FAT** | **1** | 33.2 [32.6,33.8] | 33.1 [32.7,33.5] | .830 |
| | **2** | 33.4 [32.5,34.2] | 33.6 [33.1,34.1] | .714 |
| **SFA** | **1** | 12.3 [12.0,12.6] | 12.5 [12.3,12.7] | .269 |
| | **2** | 12.2 [11.9,12.6] | 12.7 [12.4,13.0] | .097 |

CHO – total carbohydrates (% total energy); FAT – total fat (% total energy); MetS – metabolic syndrome (Alberti, 2009); SFA – saturated fatty acids (% total energy); STAR – total starch(% total energy); SUG – total sugars (% total energy); TE – total energy (kcal)

Model 1: unadjusted

Model 2: TE (kcal) total sample adjusted for age, BMI, sex, daily physical activity, number of cigarettes smoked per day

Model 2: Macronutrient intake for total sample for age, BMI, daily physical activity, number of cigarettes smoked per day and daily total energy intake

Details on the intake of total energy, carbohydrates, starch, total sugars, total fat and SFAs of the UK white male and female population are presented in Table 2.9. Healthy women consumed significantly more energy in both unadjusted ($p$<.001) and adjusted ($p$=.048) models. There were no other significant differences between those with and without MetS.

Table 2.9 – Total energy, carbohydrate and fatty acid intake in the total UK white male and female population with and without metabolic syndrome ( adjusted and unadjusted models)

| | | Men | | | Women | | |
|---|---|---|---|---|---|---|---|
| | | MetS | | | MetS | | |
| | Model | Y (n=354) | N (n=570) | p | Y (n=320) | N (n=973) | p |
| **TE** | **1** | 2110.6 [2048.0,2173.3] | 2196.6 [2119.8,2263.3] | .069 | 1492.0 [1428.2,1555.8] | 1656.3 [1618.6,1694.0] | **<.001** |
| | **2** | 2138.2 [2102.5,2270.8] | 2110.6 [2060.8,2215.5] | .450 | 1543.3 [1473.1,1613.6 | 1628.8 [1586.8,1670.9] | **.048** |
| **CHO** | **1** | 44.9 [44.0,45.8] | 45.5 [44.5,46.4] | .428 | 46.4 [45.4,47.5] | 46.3 [45.6,46.9] | .791 |
| | **2** | 45.7 [44.4,47.0] | 45.3 [44.2,46.4] | .677 | 47.7 [46.1,49.3] | 45.9 [45.1,46.7] | .068 |
| **STAR** | **1** | 25.5 24.6.,26.3] | 25.7 [25.0,26.4] | .682 | 26.2 [25.2,27.3] | 25.9 [25.3,26.4] | .594 |
| | **2** | 26.1 [24.9,27.3] | 25.7 [25.1,26.4] | .667 | 27.2 [25.7,28.7] | 25.8 [25.1,26.4] | .126 |
| **SUG** | **1** | 19.4 [18.5,20.4] | 19.8 [19.1,20.5] | .579 | 20.2 [19.1,21.3] | 20.4 [19.7,21.1] | .785 |
| | **2** | 19.6 [18.4,20.7] | 19.5 [18.6,20.4] | .949 | 20.5 [20.2,21.7] | 20.1 [19.5,20.7] | .612 |
| **FAT** | **1** | 33.2 [32.6,33.8] | 33.1 [32.7,33.5] | .830 | 33.1 [32.5,34.0] | 33.2 [32.3,33.9] | .834 |
| | **2** | 33.4 [32.5,34.2] | 33.6 [33.1,34.1] | .714 | 33.4 [32.3,34.4] | 33.6 [32.8,34.5] | .765 |
| **SFA** | **1** | 12.3 [12.0,12.6] | 12.5 [12.3,12.7] | .269 | 12.4 [11.9,12.8] | 12.4 [12.0,12.8] | .874 |
| | **2** | 12.2 [11.9,12.6] | 12.7 [12.4,13.0] | .097 | 12.4 [11.7,12.4] | 12.6 [11.8,12.4] | .480 |

CHO – total carbohydrates (% total energy); FAT – total fat (% total energy); MetS – metabolic syndrome (Alberti, 2009); SFA – saturated fatty acids (% total energy); STAR – total starch(% total energy); SUG – total sugars (% total energy); TE – total energy (kcal)
Model 1: unadjusted
Model 2: TE (kcal) for men and women adjusted for age, BMI, daily physical activity, number of cigarettes smoked per day
Model 2: Macronutrient intake for men and women adjusted for age, BMI, daily physical activity, number of cigarettes smoked per day and daily total energy intake

### 2.3.4.2 Dietary intake and cardiometabolic risk in the normal-weight, overweight and obese population with and without metabolic syndrome

Details of the dietary intake of total energy, carbohydrates and fats in those with and without MetS stratified by BMI group can be found in Table 2.10 and Table 2.11. In the unadjusted model energy intake was significantly higher in the healthy normal-weight population ($p$=.042) compared to those presenting with MetS. In the obese population intake of total starches was significantly higher in the unhealthy group ($p$=.019) after adjusting for covariates. The intake of total CHO was significantly higher in the unhealthy compared to the healthy group both before ($p$=.006) and after adjusting for covariates ($p$=.001). There were no significant differences in the intake of total fats or SFA.

Table 2.10 – Total energy and carbohydrates intake in the normal-weight, overweight and obese population with and without metabolic syndrome ( adjusted and unadjusted models)

| | **BMI** | **18.5 – 24.9** | | | **25.0 – 29.9** | | | **30.0 – 39.9** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **MetS** | | | **MetS** | | | **MetS** | | |
| | **Model** | **Y (n=76)** | **N (n=690)** | **p** | **Y (n=310)** | **N (n=537)** | **p** | **Y (n=288)** | **N (n=316)** | **p** |
| **TE** | **1** | 1767.3 [1619.9,1914.8] | 1929.3 [1878.9,1979.7] | **.042** | 1940.4 [1852.9,2027.9] | 1886.1 [1814.2,1958.0] | .326 | 1811.9 [1717.4,1906.4] | 1869.4 [1746.4,1992.3] | .467 |
| | **2** | 1839.1 [1725.0,1953.2] | 1933.3 [1878.9,1987.8] | .184 | 1906.4 1917.5,1995.4] | 1882.7 [1802.7,1962.7] | .707 | 1816.1 [1734.6,1897.6] | 1863.8 [1758.8,196.8] | .468 |
| **CHO** | **1** | 45.2 [42.8,47.7] | 46.8 [46.1,47.5] | .253 | 45.7 [44.,46.7] | 45.8 [44.9,46.6] | .956 | 45.4 [44.4,46.4] | 43.2 [41.8,44.6] | **.006** |
| | **2** | 46.1 [43.3,49.0] | 46.7 [45.9,47.4] | .846 | 46.1 [44.8,47.4] | 45.7 [44.8,46.6] | .735 | 46.0 [45.0,47.1] | 42.5 [40.9,44.2] | **.001** |
| **STAR** | **1** | 24.2 [21.9,26.5] | 25.7 [25.1,26.4] | .196 | 25.4 [24.5,26.3] | 25.8 [25.2,26.5] | .453 | 26.7 [25.7,27.7] | 25.9 [24.8,27.0] | .284 |
| | **2** | 26.5 [23.8,29.2] | 25.7 [25.1,26.4] | .788 | 26.1 [25.0,27.3] | 25.7 [24.9,26.4] | .699 | 27.4 [26.2,28.5] | 25.6 [24.3,26.8] | **.019** |
| **SUG** | **1** | 21.0 [18.8,23.3] | 21.1 [29.4,21.7] | .978 | 20.3 [19.3,21.3] | 19.9 [19.2,20.6] | .514 | 18.7 [17.6,19.9] | 17.3 16.3,18.3] | .059 |
| | **2** | 19.6 [17.2,22.0] | 20.9 [20.2,21.7] | .596 | 20.0 [18.7,21.3] | 20.0 [19.2,20.8] | .994 | 18.7 [17.5,19.8] | 17.0 [15.9,18.0] | .136 |

CHO – total carbohydrates (% total energy); MetS – metabolic syndrome (Alberti, 2009); STAR – total starch(% total energy); SUG – total sugars (% total energy); TE – total energy

Model 1: unadjusted

Model 2: TE (kcal) adjusted for age, sex, daily physical activity, number of cigarettes smoked per day

Model 2: Macronutrient intake adjusted for age, sex, physical activity, number of cigarettes smoked per day and daily total energy intake

Table 2.11 – Fatty acid intake in the normal-weight, overweight and obese population with and without metabolic syndrome ( adjusted and unadjusted models)

| | BMI | 18.5 – 24.9 MetS | | | 25.0 – 29.9 MetS | | | 30.0 – 39.9 MetS | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Model** | **Y (n=76)** | **N (n=690)** | **p** | **Y (n=310)** | **N (n=537)** | **p** | **Y (n=288)** | **N (n=316)** | **p** |
| **FAT** | **1** | 34.1 [32.5,35.7] | 33.1 [32.5,33.6] | .234 | 32.9 [32.0,33.7] | 32.9 [32.3,33.5] | .978 | 33.2 {32.2,34.2] | 33.8 [32.7,34.8] | .480 |
| | **2** | 34.2 [32.4,36.1] | 33.9 [33.3,34.5] | .794 | 32.9 [31.8,33.9] | 33.0 [32.2,33.5] | .874 | 33.3 [32.4,34.2] | 34.7 [33.3,36.1] | .193 |
| **SFA** | **1** | 12.7 [11.8,13.7] | 12.6 [12.3,12.9] | .716 | 12.1 [11.7,12.6] | 12.4 12.0,12.8] | .355 | 12.3 [11.8,12.8] | 12.5 [11.9,13.1] | .579 |
| | **2** | 12.4 [11.3,13.5] | 12.9 [12.5,13.2] | .567 | 11.9 [11.4,12.4] | 12.5 [12.1,12.9] | .086 | 12.3 [11.7,12.7] | 13.1 [12.3,13.8] | .179 |

BMI – body mass index; FAT – total fat (% total energy); MetS – metabolic syndrome (Alberti, 2009); SFA – saturated fatty acids (% total energy)
Model 1: unadjusted
Model 2: TE (kcal) adjusted for age, sex, daily physical activity, number of cigarettes smoked per day
Model 2: Macronutrient intake adjusted for age, sex, physical activity, number of cigarettes smoked per day and daily total energy intake

### 2.3.4.3 Dietary intake and cardiometabolic risk in different adult age groups with and without metabolic syndrome

Table 2.12 and Table 2.13 provide details of the total energy, carbohydrates and fat intake in those with and without MetS in the 19-34 and 35-49 age categories. In the unadjusted model energy intake was significantly higher in the unhealthy 35-49 age group ($p$=.035) compared to the healthy group. However, after adjusting for BMI, gender, daily physical activity, number of cigarettes smoked per day and total energy intake these differences were no longer significant. The intake of total starches was significantly higher in the healthy 35-49 age group compared to the unhealthy group both before ($p$=.029) and after adjusting for covariates ($p$=.033). There were no significant differences in intake of total fats and SFA between groups.

Table 2.12 – Total energy intake and dietary intake of carbohydrates in population with and without metabolic syndrome (age groups 19-34 and 35-49) – adjusted and unadjusted model

| | | 19 - 34 MetS | | | 35 - 49 MetS | | |
|---|---|---|---|---|---|---|---|
| | **Model** | **Y (n=41)** | **N (n=380)** | **P** | **Y (n=146)** | **N (n=551)** | **P** |
| **TE (kcal)** | **1** | 2038.1 [1760.5,2315.8] | 2022.8 [1949.2,2096.5] | .918 | 1999.7 [1876.1,2123.3] | 1848.0 [1780.3,1915.7] | **.035** |
| | **2** | 1757.4 [1412.8,2102.0] | 2096.0 [2014.7,2177.4] | .081 | 1963.4 [1842.7,2084.0] | 1819.3 [1752.0,1886.6] | .065 |
| **CHO** | **1** | 48.3 [45.9,50.6] | 47.2 [46.1,48.3] | .395 | 43.8 [42.0,45.7] | 45.8 [45.0,46.6] | .077 |
| | **2** | 49.6 [46.2,53.0] | 46.6 [45.3,47.9] | .123 | 44.4 [41.8,47.0] | 46.4 [45.5,46.6] | .169 |
| **STAR** | **1** | 28.8 [25.6,27.4] | 26.5 [25.6,27.3] | .140 | 25.1 [23.7,26.4] | 26.8 [26.1,27.5] | **.029** |
| | **2** | 30.2 [26.4,34.0] | 26.5 [25.6,27.4] | .066 | 25.3 [23.7,26.9] | 27.3 [26.6,28.0] | **.033** |
| **SUG** | **1** | 19.4[17.2,21.7] | 20.7 [19.8,21.5] | .312 | 18.8 [17.0,20.5] | 19.0 [18.2,19.9] | .792 |
| | **2** | 19.5 [16.1,22.8] | 20.1 [18.9,21.1] | .676 | 19.1 [18.2,20.1] | 19.1 [17.0,21.2] | .882 |

CHO – total carbohydrates (% total energy); MetS – metabolic syndrome (Alberti, 2009); STAR – total starch (% total energy); SUG – total sugars (% total energy); TE – total energy
Model 1: unadjusted
Model 2: TE (kcal) adjusted for BMI, gender, daily physical activity, number of cigarettes smoked per day
Model 2: Macronutrient intake adjusted for BMI, gender, physical activity, number of cigarettes smoked per day and daily total energy intake

Table 2.13 – Total energy intake and dietary intake of fatty acids in population with and without metabolic syndrome (age groups 19-34 and 35-49) – adjusted and unadjusted model

| | | 19 - 34 MetS | | | 35 - 49 MetS | | |
|---|---|---|---|---|---|---|---|
| | **Model** | **Y (n=41)** | **N (n=380)** | **P** | **Y (n=146)** | **N (n=551)** | **P** |
| **FAT (%TE)** | **1** | 33.2 [30.9,35.6] | 32.6 [31.8,33.4] | .668 | 33.2 [30.7,35.6] | 32.6 [31.8,33.4] | .668 |
| | **2** | 34.1 [31.0,37.2] | 34.1 [33.2,35.0] | .889 | 33.8 [32.3,35.4] | 32.7 [31.9,33.5] | .245 |
| **SFA (%TE)** | **1** | 11.9 [11.1,12.8] | 12.2 [11.8,12.3] | .598 | 12.3 [11.6,12.9] | 12.3 [11.9,12.7] | .960 |
| | **2** | 12.4 [11.0,12.8] | 12.8 [12.3,13.3] | .507 | 12.2 [11.6,12.9] | 12.0 [11.6,12.5] | .659 |

FAT – total fat (% total energy); MetS – Metabolic syndrome (Alberti, 2009); SFA – saturated fatty acids (% total energy)
Model 1: unadjusted
Model 2: TE (kcal) adjusted for BMI, gender, daily physical activity, number of cigarettes smoked per day
Model 2: Macronutrient intake adjusted for BMI, gender, physical activity, number of cigarettes smoked per day and daily total energy intake

In the ≥65 age group intake of total starches was significantly higher in the unhealthy than in the healthy group before ($p$=.008) and after adjusting for BMI, gender, daily physical activity, number of cigarettes smoked per day and total energy intake ($p$=.035). In the same age group the difference in total CHO intake between the two groups gained significance after adjusting for covariates ($p$=.028) (Table 2.14). In the 50-64 age group intake of SFA was only significantly higher for the healthy group in the unadjusted model ($p$=.004). In the adjusted model for the ≥65 age group those who were healthy consumed significantly more total fats ($p$=.019) and SFA ($p$=.043) than those who presented with MetS (Table 2.14).

Table 2.14 – Total energy intake and dietary intake of carbohydrates in population with and without metabolic syndrome (age groups 50-64 and ≥65) – adjusted and unadjusted model

| | | 50 - 64 MetS | | | ≥65 MetS | | |
|---|---|---|---|---|---|---|---|
| | **Model** | **Y (n=241)** | **N (n=390)** | **p** | **Y (n=246)** | **N (n=222)** | **P** |
| **TE (kcal)** | **1** | 1863.7 [1783.4,1944.0] | 1888.2 [1805.0,1971.2] | .677 | 1758.5 [1669.3,1847.7] | 1770.7 [1656.1,1885.4] | .875 |
| | **2** | 1872.6 [1793.1,1952.0] | 1874.9 [1786.7,1963.2] | .970 | 1706.8 [1638.9,1774.8] | 1832.5 [1721.7,1943.3] | .061 |
| **CHO** | **1** | 44.6 [43.5,45.8] | 44.5 [43.5,45.4] | .823 | 46.8 [45.7,47.8] | 45.1 [43.6,46.7] | .085 |
| | **2** | 44.8 [43.3,46.4] | 43.9 [42.9,45.0] | .632 | 47.2 [46.0,48.4] | 44.8 [43.0,46.6] | **.028** |
| **STAR** | **1** | 25.6 [24.6,26.6] | 24.5 [23.9,25.2] | .093 | 25.8 [24.6,27.0] | 23.4 [22.3,24.5] | **.008** |
| | **2** | 25.9 [24.4,27.3] | 24.6 [23.8,25.4] | .229 | 26.2 [24.9,27.6] | 23.6 [22.4,24.7] | **.035** |
| **SUG** | **1** | 19.0 [18.1,20.0] | 19.9 [19.1,20.7] | .170 | 21.0 [19.7,22.2] | 21.7 [20.6,22.8] | .341 |
| | **2** | 19.0 [17.8,20.1] | 19.3 [18.2,20.4] | .407 | 21.0 [19.9,22.1] | 21.3 [19.7,22.2] | .720 |

CHO – total carbohydrates (% total energy); MetS – metabolic syndrome (Alberti, 2009); STAR – total starch(% total energy); SUG – total sugars (% total energy); TE – total energy
Model 1: unadjusted
Model 2: TE (kcal) adjusted for BMI, gender, daily physical activity, number of cigarettes smoked per day
Model 2: Macronutrient intake adjusted for BMI, gender, physical activity, number of cigarettes smoked per day and daily total energy intake

Table 2.15 – Total energy intake and dietary intake of fatty acids in population with and without metabolic syndrome (age groups 50-64 and ≥65) – adjusted and unadjusted model

| | | 50 - 64 MetS | | | ≥65 MetS | | |
|---|---|---|---|---|---|---|---|
| | **Model** | **Y (n=241)** | **N (n=390)** | **p** | **Y (n=246)** | **N (n=222)** | **P** |
| **FAT** | **1** | 32.7 [31.7,33.7] | 33.6 [32.2,34.4] | .130 | 33.2 [32.2,34.1] | 34.1 [33.0,35.2] | .223 |
| | **2** | 33.4 [32.3,34.4] | 32.8 [32.6,34.0] | .843 | 32.7 [31.7,33.7] | 34.8 [33.0,35.2] | **.019** |
| **SFA** | **1** | 11.8 [11.3,12.2] | 12.7 [12.3,13.2] | **.004** | 12.8 [12.2,13.3] | 13.5 [12.8,14.2] | .128 |
| | **2** | 12.0 [11.5,12.6] | 12.5 [11.7,13.2] | .101 | 12.4 [11.8,13.1] | 13.8 [12.8,14.2] | **.043** |

FAT – total fat (% total energy); MetS – metabolic syndrome (Alberti, 2009); SFA – saturated fatty acids (% total energy)
Model 1: unadjusted
Model 2: TE (kcal) adjusted for BMI, gender, daily physical activity, number of cigarettes smoked per day
Model 2: Macronutrient intake adjusted for BMI, gender, physical activity, number of cigarettes smoked per day and daily total energy intake

### 2.3.5 The odds ratios of presenting with metabolic syndrome depending on dietary intake of carbohydrates and fats

#### 2.3.5.1 The odds ratios and adjusted odds ratios of presenting with metabolic syndrome depending on dietary intake of carbohydrates and fats in the total white UK population

For the total white population in the unadjusted model the odds ratio of presenting with MetS was over 30% lower in the third quartile of total fat intake compared to the fourth quartile but the significance was no longer achieved after adjusting for age, BMI, gender, number of cigarettes smoked per day, daily physical activity and total energy intake. There were significant results in the adjusted but not the unadjusted models for intake of total carbohydrates and total starches. The odds of presenting with MetS were significantly lower

in the second quartile of CHO intake and in the first and third quartile of total starch intake (Table 2.16).

Table 2.16 – Odds ratios and adjusted odds ratios of presenting with metabolic syndrome in the total white UK population depending on quartiles of macronutrient intake

| | | | Total (n=2217) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Model 1 | | | | Model 2 | | |
| | | | OR | 95% CI | | | AOR | 95% CI | | |
| | Q | %TE | | Lower | upper | p | | Lower | upper | p |
| | **1** | ≤40.8 | 1.102 | .753 | 1.614 | | .601 | .342 | 1.056 | |
| **CHO** | **2** | 40.9-45.7 | 1.201 | .856 | 1.684 | .719 | **.574** | **.361** | **.913** | .334 |
| | **3** | 45.8-50.1 | 1.170 | .807 | 1.695 | | .694 | .430 | 1.121 | |
| | **4** | ≥50.2 | **Reference category** | | | | | | | |
| | **1** | ≤21.6 | .896 | .630 | 1.275 | | **.535** | **.317** | **.904** | |
| **STAR** | **2** | 21.7-25.8 | .918 | .639 | 1.321 | .505 | .626 | .380 | 1.030 | .022 |
| | **3** | 25.9-29.9 | .763 | .536 | 1.088 | | **.489** | **.313** | **.766** | |
| | **4** | ≥30.0 | **Reference category** | | | | | | | |
| | **1** | ≤15.4 | 1.053 | .729 | 1.521 | | .863 | .507 | 1.468 | |
| **SUG** | **2** | 15.5-19.0 | .834 | .574 | 1.210 | .590 | .809 | .487 | 1.344 | .684 |
| | **3** | 19.1-23.3 | 1.010 | .680 | 1.501 | | .737 | .445 | 1.223 | |
| | **4** | ≥24.4 | **Reference category** | | | | | | | |
| | **1** | ≤29.4 | .931 | .658 | 1.319 | | 1.026 | .590 | 1.786 | |
| **FAT** | **2** | 29.5-33.2 | .927 | .641 | 1.342 | .210 | 1.166 | .720 | 1.889 | .143 |
| | **3** | 33.3-37.4 | **.668** | **.450** | **.993** | | .603 | .351 | 1.034 | |
| | **4** | ≥37.5 | **Reference category** | | | | | | | |
| | **1** | ≤10.1 | 1.157 | .822 | 1.630 | | 1.654 | .995 | 2.752 | |
| **SFA** | **2** | 10.2-12.2 | 1.003 | .695 | 1.448 | .752 | 1.620 | .922 | 2.847 | .285 |
| | **3** | 12.3-14.4 | .978 | .685 | 1.398 | | 1.269 | .761 | 2.116 | |
| | **4** | ≥14.5 | **Reference category** | | | | | | | |

AOR – adjusted odds ratio CHO – total carbohydrates (%total energy); CI – confidence interval; FAT – total fat (%total energy); OR – odds ratio; Q – quartile (4$^{th}$ quartile used as reference category); SFA – saturated fatty acids (%total energy); STAR – total starch (%total energy); SUG – total sugars (%total energy); %TE – percentage total energy

Model 1 – unadjusted

Model 2 – adjusted for age, BMI, gender, number of cigarettes smoked per day, daily physical activity, total energy intake

### 2.3.5.2 The odds ratios of presenting with metabolic syndrome depending on dietary intake of carbohydrates and fats in the white UK male population (adjusted and unadjusted models)

For white UK men the odds of presenting with MetS were double in any of the other quartiles of dietary intake of SFA compared to the highest quartile (Table 2.17).

Table 2.17 – Odds ratios and adjusted odds ratios of presenting with metabolic syndrome in the white UK male population depending on quartiles of macronutrient intake

| | | | White males only (n=924) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Model1 95% CI | | | | Model 2 95% CI | | |
| | Q | %TE | OR | Lower | upper | p | AOR | Lower | upper | p |
| CHO | **1** | ≤40.3 | 1.125 | .653 | 1.938 | .576 | .723 | .348 | 1.502 | .835 |
| | **2** | 40.4-44.7 | 1.393 | .820 | 2.367 | | .903 | .501 | 1.626 | |
| | **3** | 44.8-49.3 | 1.273 | .776 | 2.088 | | .812 | .448 | 1.473 | |
| | **4** | ≥49.4 | **Reference category** | | | | | | | |
| STAR | **1** | ≤21.6 | 1.009 | .670 | 1.520 | .227 | .644 | .363 | 1.144 | .020 |
| | **2** | 21.7-25.8 | .836 | .547 | 1.279 | | **.489** | **.290** | **.824** | |
| | **3** | 25.9-29.5 | .738 | .513 | 1.062 | | **.477** | **.295** | **.770** | |
| | **4** | ≥29.6 | **Reference category** | | | | | | | |
| SUG | **1** | ≤15.1 | 1.046 | .641 | 1.707 | .358 | .791 | .379 | 1.652 | .624 |
| | **2** | 15.2-18.4 | .819 | .498 | 1.349 | | 1.034 | .505 | 2.115 | |
| | **3** | 18.5-22.4 | .688 | .412 | 1.149 | | .672 | .362 | 1.250 | |
| | **4** | ≥22.5 | **Reference category** | | | | | | | |
| FAT | **1** | ≤29.3 | 1.022 | .620 | 1.687 | .861 | 1.036 | .495 | 2.168 | .748 |
| | **2** | 29.4-33.3 | 1.137 | .688 | 1.881 | | 1.194 | .616 | 2.313 | |
| | **3** | 33.4-37.5 | .914 | .559 | 1.495 | | .817 | .395 | 1.690 | |
| | **4** | ≥37.6 | **Reference category** | | | | | | | |
| SFA | **1** | ≤10.1 | 1.340 | .836 | 2.150 | .503 | **2.002** | **1.029** | **3.895** | .111 |
| | **2** | 10.2-12.3 | 1.135 | .719 | 1.790 | | **2.008** | **1.050** | **3.840** | |
| | **3** | 12.4-14.2 | 1.321 | .855 | 2.041 | | **2.014** | **1.036** | **3.914** | |
| | **4** | ≥14.3 | **Reference category** | | | | | | | |

AOR – adjusted odds ratio CHO – total carbohydrates (%total energy); CI – confidence interval; FAT – total fat (%total energy); Q – quartile (4th quartile used as reference category); SFA – saturated fatty acids (%total energy); STAR – total starch (%total energy); SUG – total sugars (%total energy); %TE – percentage total energy

Model 1 – unadjusted

Model 2 – adjusted for age, BMI, number of cigarettes smoked per day, daily physical activity, total energy intake

### 2.3.5.3 The odds ratios and adjusted odds ratios of presenting with metabolic syndrome depending on dietary intake of carbohydrates and fats in the white UK female population

For the white UK female population, the unadjusted odds of presenting with MetS were 40% lower in the third quartile of total fat intake compared to the fourth. The odds were also lower in the other intake quartiles, but the range of the 95%CI did not demonstrate significance. After adjusting for covariates, the AOR for total fat intake the range of the 95%CI still demonstrated significance in the third quartile and was smaller compared to the unadjusted OR. However, the increase in the range of the 95% CI points for this result were less precise. The results obtained for intake of CHO and starches showed significance in the adjusted models. Those in the lowest intake quartile and the second lowest intake quartile presented with up to 60% reduced odds of having MetS. Those in the lowest intake quartile and the second highest intake presented with up to 66% reduced odds of having MetS. (Table 2.18).

Table 2.18 – Odds ratios and adjusted odds ratios of presenting with metabolic syndrome in the white UK female population depending on quartiles of macronutrient intake

| | | | White females only (n=1293) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Model 1 | | | | Model 2 | | |
| | | | OR | 95% CI | | | AOR | 95% CI | | |
| | Q | %TE | | Lower | upper | p | | Lower | upper | p |
| CHO | **1** | ≤41.3 | .878 | .548 | 1.408 | .781 | **.441** | **.215** | **.903** | .161 |
| | **2** | 41.4-46.3 | .823 | .531 | 1.275 | | **.390** | **.207** | **.736** | |
| | **3** | 46.4-50.6 | .793 | .493 | 1.275 | | .562 | .298 | 1.059 | |
| | **4** | ≥50.7 | **Reference category** | | | | | | | |
| STAR | **1** | ≤21.5 | .721 | .430 | 1.206 | .307 | **.345** | **.174** | **.683** | .005 |
| | **2** | 21.6-25.7 | 1.024 | .630 | 1.664 | | .951 | .557 | 1.625 | |
| | **3** | 25.8-30.2 | .733 | .461 | 1.166 | | **.485** | **.236** | **.995** | |
| | **4** | ≥30.3 | **Reference category** | | | | | | | |
| SUG | **1** | ≤15.6 | .988 | .635 | 1.536 | .064 | .971 | .527 | 1.788 | .051 |
| | **2** | 15.7-19.5 | .703 | .447 | 1.107 | | .566 | .305 | 1.050 | |
| | **3** | 19.6-23.8 | 1.215 | .754 | 1.958 | | .626 | .284 | 1.376 | |
| | **4** | ≥23.9 | **Reference category** | | | | | | | |
| FAT | **1** | ≤29.4 | .950 | .620 | 1.455 | .019 | .996 | .540 | 1.838 | .241 |
| | **2** | 29.5-33.2 | .773 | .487 | 1.226 | | .940 | .546 | 1.618 | |
| | **3** | 33.3-37.5 | **.604** | **.414** | **.882** | | **.498** | **.253** | **.980** | |
| | **4** | ≥37.6 | **Reference category** | | | | | | | |
| SFA | **1** | ≤10.2 | 1.362 | .886 | 2.094 | .540 | 1.693 | .898 | 3.193 | .414 |
| | **2** | 10.3-12.1 | 1.040 | .678 | 1.595 | | 1.208 | .714 | 2.043 | |
| | **3** | 12.2-14.5 | 1.057 | .706 | 1.583 | | .939 | .453 | 1.948 | |
| | **4** | ≥14.6 | **Reference category** | | | | | | | |

AOR – adjusted odds ratio CHO – total carbohydrates (%total energy); CI – confidence interval; FAT – total fat (%total energy); OR – odds ratio; Q – quartile (4th quartile used as reference category); SFA – saturated fatty acids (%total energy); STAR – total starch (%total energy); SUG – total sugars (%total energy); %TE – percentage total energy

Model 1 – unadjusted

Model 2 – adjusted for age, BMI, number of cigarettes smoked per day, daily physical activity, total energy intake

### 2.3.5.4 The odds ratios and adjusted odds ratios of presenting with metabolic syndrome depending on dietary intake of carbohydrates and fats in the white UK normal-weight population

In the UK white normal-weight population there were some significantly reduced odds of presenting with MetS with lower intake levels for SUG, FAT and SFA. However, after adjusting for covariates these no longer retained their significance. After adjustments intakes for the second quartile of total CHO and the first quartile of SUG did gain significance. The odds of presenting with MetS were nearly 70% lower in the second quartile of CHO intake. The odds of presenting with MetS were increased by nearly 80% in the lowest quartile of total sugar intake compared to the reference category (Table 2.19).

Table 2.19 – Odds ratios and adjusted odds ratios of presenting with metabolic syndrome in the white UK normal-weight population depending on quartiles of macronutrient intake

| | | | BMI 18.5 – 24.9 kg/m$^2$ (n=766) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Model 1 95% CI | | | | Model 2 95% CI | | |
| | Q | %TE | OR | Lower | upper | p | AOR | Lower | upper | p |
| **CHO** | **1** | ≤41.5 | 1.021 | .605 | 1.723 | .317 | 1.275 | .698 | 2.328 | .001 |
| | **2** | 41.6-46.2 | .793 | .485 | 1.295 | | **.313** | **.151** | **.650** | |
| | **3** | 46.3-49.9 | .742 | .414 | 1.333 | | .735 | .381 | 1.416 | |
| | **4** | ≥50.0 | **Reference category** | | | | | | | |
| **STAR** | **1** | ≤21.2 | 1.436 | .853 | 2.418 | .034 | .742 | .372 | 1.482 | .847 |
| | **2** | 21.3-25.2 | 1.329 | .811 | 2.176 | | .589 | .268 | 1.293 | |
| | **3** | 25.3-29.4 | .409 | .153 | 1.094 | | .588 | .198 | 1.743 | |
| | **4** | ≥29.5 | **Reference category** | | | | | | | |
| **SUG** | **1** | ≤16.2 | .897 | .527 | 1.524 | .008 | **1.798** | **1.020** | **3.168** | .005 |
| | **2** | 16.3-19.9 | **.569** | **.349** | **.927** | | .974 | .487 | 1.947 | |
| | **3** | 20.0-24.4 | **.464** | **.230** | **.935** | | .462 | .184 | 1.161 | |
| | **4** | ≥24.5 | **Reference category** | | | | | | | |
| **FAT** | **1** | ≤29.9 | .780 | .506 | 1.201 | <.0001 | 1.055 | .491 | 2.265 | .460 |
| | **2** | 30.0-33.5 | .916 | .501 | 1.675 | | .829 | .339 | 2.026 | |
| | **3** | 33.6-37.5 | **.503** | **.395** | **.640** | | .713 | .331 | 1.534 | |
| | **4** | ≥37.6 | **Reference category** | | | | | | | |
| **SFA** | **1** | ≤10.3 | .843 | .498 | 1.425 | .049 | 1.410 | .645 | 3.082 | .253 |
| | **2** | 10.4-12.3 | .727 | .444 | 1.189 | | .850 | .398 | 1.817 | |
| | **3** | 12.4-14.6 | **.488** | **.290** | **.822** | | .559 | .245 | 1.278 | |
| | **4** | ≥14.7 | **Reference category** | | | | | | | |

AOR – adjusted odds ratio CHO – total carbohydrates (%total energy); CI – confidence interval; FAT – total fat (%total energy); OR – odds ratio; Q – quartile (4$^{th}$ quartile used as reference category); SFA – saturated fatty acids (%total energy); STAR – total starch (%total energy); SUG – total sugars (%total energy); %TE – percentage total energy

Model 1 – unadjusted

Model 2 – adjusted for age, gender, number of cigarettes smoked per day, daily physical activity, total energy intake

### 2.3.5.5 The odds ratios and adjusted odds ratios of presenting with metabolic syndrome depending on dietary intake of carbohydrates and fats in the white UK overweight population

There were no significant findings regarding dietary intake of carbohydrates and fatty acids and potential increases or decreases in odds ratios of presenting with MetS in the UK white overweight population. All CIs for unadjusted and adjusted models for all macronutrients crossed the null value (Table 2.20).

Table 2.20 – Odds ratios and adjusted odds ratios of presenting with metabolic syndrome in the white UK overweight population depending on quartiles of macronutrient intake

| | | | BMI 25.0 – 29.9 kg/m$^2$ (n=847) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Model 1 | | | | Model 2 | | |
| | | | OR | 95% CI | | | AOR | 95% CI | | |
| | Q | %TE | | Lower | upper | p | | Lower | upper | p |
| **CHO** | **1** | ≤40.6 | 1.014 | .644 | 1.596 | .887 | .827 | .495 | 1.381 | .452 |
| | **2** | 40.7-45.6 | .858 | .543 | 1.354 | | .577 | .333 | 1.001 | |
| | **3** | 45.7-50.0 | .928 | .598 | 1.440 | | .794 | .461 | 1.368 | |
| | **4** | ≥50.1 | **Reference category** | | | | | | | |
| **STAR** | **1** | ≤21.4 | 1.117 | .676 | 1.846 | .665 | .489 | .235 | 1.019 | .400 |
| | **2** | 21.5-25.7 | 1.099 | .688 | 1.756 | | .738 | .374 | 1.456 | |
| | **3** | 25.8-29.6 | 1.251 | .867 | 1.806 | | .712 | .416 | 1.217 | |
| | **4** | ≥29.7 | **Reference category** | | | | | | | |
| **SUG** | **1** | ≤15.6 | .686 | .418 | 1.127 | .367 | .962 | .502 | 1.844 | .652 |
| | **2** | 15.7-18.9 | .763 | .493 | 1.180 | | .785 | .407 | 1.515 | |
| | **3** | 19.0-23.1 | .938 | .647 | 1.358 | | .706 | .415 | 1.201 | |
| | **4** | ≥23.2 | **Reference category** | | | | | | | |
| **FAT** | **1** | ≤29.3 | .800 | .490 | 1.305 | .037 | 1.010 | .524 | 1.943 | .691 |
| | **2** | 29.4-33.2 | 1.124 | .694 | 1.820 | | 1.327 | .679 | 2.591 | |
| | **3** | 33.3-37.2 | .637 | .383 | 1.060 | | .823 | .431 | 1.570 | |
| | **4** | ≥37.3 | **Reference category** | | | | | | | |
| **SFA** | **1** | ≤10.2 | 1.338 | .823 | 2.175 | .312 | 1.893 | .981 | 3.654 | .329 |
| | **2** | 103-12.2 | .810 | .471 | 1.391 | | 1.075 | .513 | 2.255 | |
| | **3** | 12.3-14.4 | .997 | .606 | 1.641 | | 1.256 | .635 | 2.487 | |
| | **4** | ≥14.5 | **Reference category** | | | | | | | |

AOR – adjusted odds ratio CHO – total carbohydrates (%total energy); CI – confidence interval; FAT – total fat (%total energy); OR – odds ratio; Q – quartile (4$^{th}$ quartile used as reference category); SFA – saturated fatty acids (%total energy); STAR – total starch (%total energy); SUG – total sugars (%total energy); %TE – percentage total energy

Model 1 – unadjusted

Model 2 – adjusted for age, gender, number of cigarettes smoked per day, daily physical activity, total energy intake

### 2.3.5.6 The odds ratios and adjusted odds ratios of presenting with metabolic syndrome depending on dietary intake of carbohydrates and fats in the white UK obese population

For the white UK obese population there were a number of significant findings regarding dietary intake and the odds presenting with MetS. All the significant results in the unadjusted model retained their significance after adjusting for covariates in the model. Both the unadjusted and adjusted odds of presenting with MetS were lower in the first and the third quartile of total CHO intake and lower in the second quartile of total sugar intake. After adjusting for covariates, the odds increased, and the 95% CIs decreased, showing that these results gain in precision. The odds of presenting with MetS were significantly higher in the third quartile of SFA intake but although the significance was retained after adjusting for covariates and the odds increased nearly ten-fold the level of precision decreased. After adjusting for covariates the odds of presenting with Mets became significant for some quartiles of dietary intake in the UK white obese population. Those in the first and third quartile of total sugar intake, in the third quartile of total fat intake and the first quartile of SFA intake had a significantly decreased risk of presenting with MetS. This risk was also significantly decreased in those in the second quartile of total fat intake. However, the extremely low values raise questions of the validity of the overall adjusted model, which might have been due to the model being over-fitted in this particular case. Likewise, the 95% CI for adjusted SFA intake was largely demonstrating low levels of precision (Table 2.21).

Table 2.21 – Odds ratio and adjusted odds ratios of presenting with metabolic syndrome in the white UK obese population depending on quartiles of macronutrient intake

| | | | BMI 30.0 – 39.9 kg/m$^2$ (n=604) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Model 1 95% CI | | | | Model 2 95% CI | | |
| | Q | %TE | OR | Lower | upper | p | AOR | Lower | upper | p |
| **CHO** | **1** | ≤40.3 | **.425** | **.275** | **.657** | .001 | **.194** | **.095** | **.399** | .001 |
| | **2** | 40.4-45.1 | .788 | .469 | 1.324 | | .832 | .264 | 2.620 | |
| | **3** | 45.2-49.6 | **.595** | **.380** | **.930** | | **.372** | **.202** | **.688** | |
| | **4** | ≥49.7 | **Reference category** | | | | | | | |
| **STAR** | **1** | ≤22.3 | .743 | .434 | 1.272 | .528 | .557 | .270 | 1.149 | .012 |
| | **2** | 22.4-26.4 | .795 | .514 | 1.229 | | 1.006 | .702 | 1.441 | |
| | **3** | 26.5-30.8 | .974 | .633 | 1.497 | | 1.256 | .606 | 2.603 | |
| | **4** | ≥30.9 | **Reference category** | | | | | | | |
| **SUG** | **1** | ≤14.1 | .793 | .486 | 1.294 | <.0001 | **.258** | **.094** | **.710** | .016 |
| | **2** | 14.2-17.9 | **.481** | **.286** | **.811** | | **.180** | **.070** | **.465** | |
| | **3** | 18.0-21.7 | 1.432 | .909 | 2.257 | | **.401** | **.162** | **.997** | |
| | **4** | ≥21.8 | **Reference category** | | | | | | | |
| **FAT** | **1** | ≤29.0 | .953 | .658 | 1.380 | .254 | 1.233 | .413 | 3.688 | .014 |
| | **2** | 29.1-33.2 | .869 | .534 | 1.412 | | **7.680$^{E+41}$** | **3.722$^{E+41}$** | **1.585$^{E+43}$** | |
| | **3** | 33.3-37.6 | .667 | .443 | 1.003 | | **.466** | **.273** | **.796** | |
| | **4** | ≥37.7 | **Reference category** | | | | | | | |
| **SFA** | **1** | ≤9.9 | 1.224 | .839 | 1.786 | .016 | **7.306** | **1.329** | **40.177** | .086 |
| | **2** | 10.0-12.0 | 1.008 | .709 | 1.434 | | .500 | .116 | 2.151 | |
| | **3** | 12.1-14.1 | **1.959** | **1.289** | **2.979** | | **19.804** | **6.324** | **62.014** | |
| | **4** | ≥14.2 | **Reference category** | | | | | | | |

AOR – adjusted odds ratio CHO – total carbohydrates (%total energy); CI – confidence interval; FAT – total fat (%total energy); OR – odds ratio; Q – quartile (4$^{th}$ quartile used as reference category); SFA – saturated fatty acids (%total energy); STAR – total starch (%total energy); SUG – total sugars (%total energy); %TE – percentage total energy

Model 1 – unadjusted

Model 2 – adjusted for age, gender, number of cigarettes smoked per day, daily physical activity, total energy intake

### 2.3.5.7 The odds ratios and adjusted odds ratios of presenting with metabolic syndrome depending on dietary intake of carbohydrates and fats in the white UK population aged 19-34 years

In the UK white population aged 19-34 years there were a number of significant findings in both the unadjusted and the adjusted models. The odds of presenting with MetS were significantly lower in the first quartile of total CHO intake, the first and the third quartile of total starch intake and significantly higher in the first and the third quartile of SFA intake compared to the highest quartiles. After adjusting for covariates, the odds for the first quartile of total CHO intake decreased and the level of precision increased. However, the level of precision for the findings for SFA intake decreased. Only after adjusting for confounding factors were there significant findings also for the first quartile of total sugar and the first quartile of total fat intake (Table 2.22).

Table 2.22 – Odds ratios and adjusted odds ratios of presenting with metabolic syndrome in the white UK population aged 19-34 years depending on quartiles of macronutrient intake

| | | | Age group 19 – 34 years (n=421) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Model 1 95% CI | | | | Model 2 95% CI | | |
| | Q | %TE | OR | Lower | upper | p | AOR | Lower | upper | p |
| **CHO** | **1** | ≤42.2 | **.463** | **.255** | **.839** | <.0001 | **.150** | **.090** | **.252** | <.001 |
| | **2** | 42.3-47.2 | 1.139 | .469 | 2.767 | | .547 | .145 | 2.057 | |
| | **3** | 47.3-51.2 | .999 | .214 | 4.660 | | .670 | .192 | 2.339 | |
| | **4** | ≥51.3 | **Reference category** | | | | | | | |
| **STAR** | **1** | ≤22.4 | **.241** | **.108** | **.538** | <.0001 | **.124** | **.034** | **.457** | <.001 |
| | **2** | 22.5-26.5 | 1.541 | .477 | 4.973 | | 2.911 | .803 | 10.559 | |
| | **3** | 26.6.-30.4 | **.356** | **.147** | **.862** | | **.254** | **.085** | **.755** | |
| | **4** | ≥30.5 | **Reference category** | | | | | | | |
| **SUG** | **1** | ≤16.3 | .877 | .232 | 3.314 | .423 | **.204** | **.052** | **.801** | .170 |
| | **2** | 19.4 | .951 | .199 | 4.546 | | 1.201 | .268 | 5.378 | |
| | **3** | 19.5-23.7 | 1.311 | .460 | 3.740 | | 1.268 | .329 | 4.886 | |
| | **4** | ≥23.8 | **Reference category** | | | | | | | |
| **FAT** | **1** | ≤29.4 | .664 | .278 | 1.586 | .286 | **.255** | **.110** | **.592** | .156 |
| | **2** | 29.5-33.0 | 1.185 | .294 | 4.771 | | 1.427 | .271 | 7.517 | |
| | **3** | 33.1-36.9 | .737 | .351 | 1.550 | | .903 | .228 | 3.579 | |
| | **4** | ≥37.9 | **Reference category** | | | | | | | |
| **SFA** | **1** | ≤10.0 | **3.108** | **1.268** | **7.622** | <.0001 | **3.970** | **1.118** | **14.094** | .002 |
| | **2** | 10.1-11.7 | 2.030 | .781 | 5.275 | | **.000** | **.000** | **.000** | |
| | **3** | 12.1-14.1 | **1.959** | **1.289** | **2.979** | | **19.804** | **6.324** | **62.014** | |
| | **4** | ≥14.2 | **Reference category** | | | | | | | |

AOR – adjusted odds ratio CHO – total carbohydrates (%total energy); CI – confidence interval; FAT – total fat (%total energy); OR – odds ratio; Q – quartile ($4^{th}$ quartile used as reference category); SFA – saturated fatty acids (%total energy); STAR – total starch (%total energy); SUG – total sugars (%total energy); %TE – percentage total energy; Model 1 – unadjusted; Model 2 – adjusted for BMI, gender, number of cigarettes smoked per day, daily physical activity, total energy intake

### 2.3.5.8 The odds ratios and adjusted odds ratios of presenting with metabolic syndrome depending on dietary intake of carbohydrates and fats in the white UK population aged 35-49 years

In the white UK population aged 35-49 years the odds of presenting with MetS were significantly higher in both unadjusted and adjusted models in the first quartile of total starch intake and the third quartile of SFA intake. Before adjusting for confounding factors those being in the first quartile of total CHO intake and the third quartile of total starch intake had significantly increased odds of presenting with MetS. Only in the adjusted model had those in the first quartile of SFA intake increased odds of presenting with MetS (Table 2.23).

Table 2.23 – Odds ratios and adjusted odds ratios of presenting with metabolic syndrome in the UK white population aged 35-49 years depending on quartiles of macronutrient intake

| | | | Age group 35 - 49 years (n=697) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Model 1 95% CI | | | | Model 2 95% CI | | |
| | Q | %TE | OR | Lower | upper | p | AOR | Lower | upper | p |
| **CHO** | **1** | ≤40.7 | **2.037** | **1.049** | **3.956** | .001 | 1.668 | .766 | 3.631 | <.009 |
| | **2** | 40.8-45.5 | 1.660 | .843 | 3.272 | | 1.150 | .579 | 2.285 | |
| | **3** | 45.6-49.9 | .811 | .460 | 1.432 | | .632 | .342 | 1.169 | |
| | **4** | ≥50.0 | **Reference category** | | | | | | | |
| **STAR** | **1** | ≤22.3 | **2.387** | **1.429** | **3.986** | <.0001 | **2.136** | **1.047** | **4.357** | .135 |
| | **2** | 22.4-26.5 | .904 | .526 | 1.554 | | .890 | .415 | 1.908 | |
| | **3** | 26.4-30.7 | **1.637** | **1.061** | **2.526** | | 1.502 | .864 | 2.610 | |
| | **4** | ≥30.8 | **Reference category** | | | | | | | |
| **SUG** | **1** | ≤14.6 | 1.198 | .683 | 2.102 | .001 | .947 | .437 | 2.054 | .541 |
| | **2** | 14.7-18.0 | .771 | .372 | 1.597 | | .560 | .216 | 1.455 | |
| | **3** | 18.1-22.2 | .749 | .420 | 1.335 | | .560 | .278 | 1.127 | |
| | **4** | ≥23.3 | **Reference category** | | | | | | | |
| **FAT** | **1** | ≤29.2 | **.481** | **.278** | **.831** | .001 | .587 | .296 | 1.161 | .138 |
| | **2** | 29.3-33.3 | .742 | .403 | 1.366 | | .973 | .511 | 1.849 | |
| | **3** | 33.4-37.5 | **.347** | **.169** | **.709** | | .480 | .216 | 1.067 | |
| | **4** | ≥37.6 | **Reference category** | | | | | | | |
| **SFA** | **1** | ≤9.9 | 1.311 | .733 | 2.342 | .150 | **2.081** | **1.131** | **3.830** | .004 |
| | **2** | 10.0-12.0 | 1.115 | .614 | 2.026 | | 1.049 | .606 | 1.819 | |
| | **3** | 12.1-14.1 | **1.891** | **1.068** | **3.349** | | **2.768** | **1.494** | **5.126** | |
| | **4** | ≥14.2 | **Reference category** | | | | | | | |

AOR – adjusted odds ratio CHO – total carbohydrates (%total energy); CI – confidence interval; FAT – total fat (%total energy); OR – odds ratio; Q – quartile (4th quartile used as reference category); SFA – saturated fatty acids (%total energy); STAR – total starch (%total energy); SUG – total sugars (%total energy); %TE – percentage total energy

Model 1 – unadjusted

Model 2 – adjusted for BMI, gender, number of cigarettes smoked per day, daily physical activity, total energy intake

### 2.3.5.9 The odds ratios and adjusted odds ratios of presenting with metabolic syndrome depending on dietary intake of carbohydrates and fats in the white UK population aged 50-64 years

In the white UK population aged 50-64 years in both unadjusted and adjusted models those in the first quartile of total sugar intake and those in the second quartile of SFA intake had significantly higher odds of presenting with MetS, whilst those in the second quartile of total starch intake had significantly lower odds for this. Whilst the level of precision increased for starch intake in the second quartile, the precision for both SFA and total sugar was lower in the adjusted model. Level of precision was considerably lower for the adjusted SFA model. The odds of presenting with MetS were higher for the first quartile of SFA intake in the unadjusted model only. After adjusting for confounding factors the odds of presenting with MetS were significantly lower for the first, the second and the third quartile of CHO intake. Levels of precision were particularly high for CHO intake due to the small CIs (Table 2.24).

Table 2.24 – Odds ratios and adjusted odds ratios of presenting with metabolic syndrome in the white UK population aged 50-64 years depending on quartiles of macronutrient intake

| | | | Age group 50 - 64 years only (n=631) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Model 1 95% CI | | | | Model 2 95% CI | | | |
| | Q | %TE | OR | Lower | upper | p | AOR | Lower | upper | p |
| **CHO** | **1** | ≤39.7 | .944 | .585 | 1.526 | .240 | **.036** | **.010** | **.126** | <.0001 |
| | **2** | 39.8-44.6 | .691 | .452 | 1.059 | | **.046** | **.015** | **.143** | |
| | **3** | 44.7-49.6 | .732 | .486 | 1.102 | | **.020** | **.006** | **.068** | |
| | **4** | ≥49.7 | **Reference category** | | | | | | | |
| **STAR** | **1** | ≤20.9 | .682 | .437 | 1.065 | .055 | .724 | .462 | 1.134 | .158 |
| | **2** | 21.0-25.0 | **.575** | **.367** | **.900** | | **.383** | **.215** | **.681** | |
| | **3** | 25.1-29.3 | .827 | .505 | 1.354 | | .731 | .395 | 1.353 | |
| | **4** | ≥29.4 | **Reference category** | | | | | | | |
| **SUG** | **1** | ≤14.9 | **1.822** | **1.133** | **2.930** | .019 | **2.039** | **1.168** | **3.561** | <.0001 |
| | **2** | 15.0-18.8 | .828 | .499 | 1.375 | | .791 | .389 | 1.607 | |
| | **3** | 18.9-23.3 | 1.029 | .685 | 1.545 | | 1.910 | .763 | 4.779 | |
| | **4** | ≥23.4 | **Reference category** | | | | | | | |
| **FAT** | **1** | ≤29.0 | 1.310 | .868 | 1.978 | <.0001 | .962 | .664 | 1.393 | .011 |
| | **2** | 29.1-33.0 | 1.316 | .914 | 1.894 | | 1.859 | .776 | 4.451 | |
| | **3** | 33.1-37.1 | **.509** | **.339** | **.765** | | .804 | .358 | 1.804 | |
| | **4** | ≥37.2 | **Reference category** | | | | | | | |
| **SFA** | **1** | ≤9.9 | **1.882** | **1.326** | **2.670** | .001 | 1.100 | .696 | 1.738 | .137 |
| | **2** | 10.0-12.2 | **1.920** | **1.312** | **2.811** | | **7.195** | **1.076** | **48.123** | |
| | **3** | 12.3-14.5 | 1.040 | .699 | 1.547 | | .834 | .490 | 1.417 | |
| | **4** | ≥14.6 | **Reference category** | | | | | | | |

AOR – adjusted odds ratio CHO – total carbohydrates (%total energy); CI – confidence interval; FAT – total fat (%total energy); OR – odds ratio; Q – quartile (4th quartile used as reference category); SFA – saturated fatty acids (%total energy); STAR – total starch (%total energy); SUG – total sugars (%total energy); %TE – percentage total energy

Model 1 – unadjusted

Model 2 – adjusted for BMI, gender, number of cigarettes smoked per day, daily physical activity, total energy intake

### 2.3.5.10 The odds ratios and adjusted odds ratios of presenting with metabolic syndrome depending on dietary intake of carbohydrates and fats in the white UK population aged ≥65years

In the white UK population aged ≥65 years the odds of presenting with MetS were significantly lower in both the unadjusted and the adjusted model for the first quartile of total CHO intake, the first, second and third quartiles of total starch intake, and significantly higher for the first quartile of total fat intake. In the unadjusted model odds of presenting with MetS were also higher for the first quartile of SFA intake but after adjusting for potential confounders the significance was not retained. In addition, in the adjusted model intake of CHO for the second quartile and of total sugars in the third quartile meant significantly lower odds of presenting with MetS. However, for the adjusted third quartile of total sugar intake values were either extremely low or extremely low so that caution needs to be applied for the interpretation of these results (Table 2.25).

Table 2.25 – Odds ratios and adjusted odds ratios of presenting with metabolic syndrome in the UK white population aged ≥65 years depending on quartiles of macronutrient intake

| | | | Age group ≥65 years (n=468) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Model 1 95% CI | | | | Model 2 95% CI | | | |
| | Q | %TE | OR | Lower | upper | p | AOR | Lower | upper | p |
| **CHO** | **1** | ≤41.6 | **.455** | **.264** | **.785** | <.0001 | **.218** | **.094** | **.506** | <.0001 |
| | **2** | 41.7-46.2 | .711 | .395 | 1.279 | | **.434** | **.245** | **.767** | |
| | **3** | 46.3-50.1 | 1.246 | .758 | 2.048 | | 1.095 | .597 | 2.008 | |
| | **4** | ≥50.2 | **Reference category** | | | | | | | |
| **STAR** | **1** | ≤20.9 | **.290** | **.139** | **.603** | .016 | **.173** | **.074** | **.401** | .010 |
| | **2** | 21.0-24.9 | **.429** | **.231** | **.799** | | **.293** | **.130** | **.662** | |
| | **3** | 25.0-28.7 | **.504** | **.300** | **.849** | | **.245** | **.132** | **.455** | |
| | **4** | ≥28.8 | **Reference category** | | | | | | | |
| **SUG** | **1** | ≤16.4 | 1.198 | .705 | 2.037 | .141 | 1.009 | .493 | 2.064 | .136 |
| | **2** | 16.5-20.4 | 1.119 | .791 | 1.583 | | .613 | .358 | 1.051 | |
| | **3** | 20.5-24.4 | .760 | .413 | 1.397 | | **.001** | **$9.872^{E-5}$** | **.004** | |
| | **4** | ≥24.5 | **Reference category** | | | | | | | |
| **FAT** | **1** | ≤30.1 | **1.559** | **1.029** | **2.363** | .107 | **1.994** | **1.276** | **3.117** | .064 |
| | **2** | 30.2-33.7 | 1.213 | .744 | 1.980 | | 1.256 | .735 | 2.148 | |
| | **3** | 33.8-38.0 | 1.279 | .776 | 2.109 | | 1.508 | .831 | 2.735 | |
| | **4** | ≥38.1 | **Reference category** | | | | | | | |
| **SFA** | **1** | ≤10.8 | **1.940** | **1.067** | **3.528** | .129 | 2.749 | .844 | 8.951 | .021 |
| | **2** | 10.9-12.9 | 1.455 | .905 | 2.341 | | 2.246 | .532 | 9.472 | |
| | **3** | 13.0-15.2 | 1.153 | .691 | 1.926 | | 1.508 | .493 | 4.611 | |
| | **4** | ≥15.3 | **Reference category** | | | | | | | |

AOR – adjusted odds ratio CHO – total carbohydrates (%total energy); CI – confidence interval; FAT – total fat (%total energy); OR – odds ratio; Q – quartile ($4^{th}$ quartile used as reference category); SFA – saturated fatty acids (%total energy); STAR – total starch (%total energy); SUG – total sugars (%total energy); %TE – percentage total energy

Model 1 – unadjusted

Model 2 – adjusted for BMI, gender, number of cigarettes smoked per day, daily physical activity, total energy intake

## 2.4 Discussion

To the candidate's knowledge, this is the first study to investigate potential associations between dietary CHO and fat intake and the odds of presenting with MetS in the UK white population by undertaking secondary analysis of NDNS data. It is also one of the few to analyse the data using the complex sample mode (Mak et al., 2013), which is deemed to be the appropriate method for analysing data sets that have been designed to be representative of a population, such as the NDNS (Saylor et al., 2012; Zumaeta et al., 2016).

The initial analysis showed only a few significant differences in dietary intake of CHO and fats between those with or without MetS when the data was analysed for the total sample or stratified by gender or by BMI group. The most significant differences occurred in the ≥65 age group. For the majority of findings differences were no longer significant after adjusting for confounding factors known to have an impact on CM risk, namely age, BMI, gender, smoking and physical activity levels (World Heart Federation, 2017). However, especially in the ≥65 age group the differences in total CHO, STAR, total fat and SFA intake gained significance after adjusting for covariates. For the majority of significant findings total CHO, STAR and SUG was higher in those presenting with MetS, whereas intake of total fats and SFA was lower. Mean estimate intake of total CHO was lower than the recommended 50% of total energy in any of the stratified data analysed but SFA intake was higher than the recommended 10% of total energy, even in those free of MetS.

The logistic regression analyses showed that there was not necessarily a linear relationship between quartiles of dietary intake and the odds of presenting with MetS, even in cases where findings were significant for all quartiles. For example whilst in the white obese population all of the first three quartiles of total sugar intake had lower odds of presenting with MetS compared to the highest quartile, with those in the second quartile (rather than the first quartile) that presented with the lowest odds (an 82% reduction) of having MetS (Table 2.22). Likewise, the adjusted odds for white males of presenting with MetS were double in any of the first three quartiles of SFA intake compared to those in the highest intake category but there was no further differentiation of odds between the first three quartiles (Table 2.18). Being in the lowest quartile of dietary intake did not necessarily mean the highest or lowest odds of presenting with MetS. In the total UK white population and in those in the 50-64 age group it were actually those in the second quartile of STAR intake who had the lowest odds of presenting with MetS (Table 2.25).

Interestingly, the lowest and the highest percentile thresholds did not show extreme values for CHO, total fat and SFA intake. The lowest value of the upper limit for the first quartile

of CHO intake was ≤39.7 %TE and occurred in the 50-64 age group (Table 2.25), which would be classed as a moderately low or reduced-CHO diet (Guess, 2017; Wylie-Rosett et al., 2013). The highest value of the upper limit for the first quartile of CHO intake was ≤42.3 %TE (moderate-CHO diet (Noakes and Windt, 2017)) and occurred in the 19-34 age group (Table 2.23). Further examples of lacks of extremes can be found across the Tables.

The majority of those within the lowest quartile therefore consumed what would be classed as a low-fat diet (Nordmann et al., 2011), whilst those in the highest quartile would mostly still be within the moderate-fat range. A large majority of those in any of the lowest quartile of SFA intake meet current UK recommendations of not exceeding 10% of total energy for SFA intake, whilst all of those in the highest quartile would mostly be exceeding those recommendations.

Overall, the majority of findings from the present analysis showed that moderate or even reduced intake of total CHO, and lower intake of STAR and SUG decreased the odds of presenting with MetS, whereas following a low-fat diet and adhering to the guidelines for SFA intake increased the odds significantly. Whilst gender stratification only produced a very limited number of significant results, interestingly most of these occurred after adjusting for confounding factors. In the white obese UK population intake of total CHO, SUG and SFA findings were significant in both the unadjusted and adjusted model. The odds of presenting with MetS were significantly lower with lower CHO and lower SUG intakes, but significantly higher with lower SFA intake. The most consistent findings were made when the analysis was stratified by age group when, even after adjusting for confounding factors, most results for total CHO, STAR, SUG, total fats and SFA intake either retained their significance or gained significance across nearly all age groups. The results here also showed that for intake of total CHO or individial subtypes of CHO the odds of presenting with MetS decreased with lower intakes, whereas these odds increased with lower total fat or SFA intake. These are interesting findings that add to the debate about whether high CHO intake is beneficial and challenge the assumptions that SFA intake is detrimental to CM health (see chapter 1.0).

To the author's knowledge only one study to date investigated the diet composition of those obtaining less than 40% TE from CHO in the general UK population. Guess (2017) analysed the data of 2263 individuals aged 16-75 years in the NDNS RP 2008-2012. Of these n=430 reported CHO intakes below the 40%TE threshold. Mean CHO intake was 35.3%TE. This study however did not use complex survey analysis but parametric and non-parametric tests applicable for a random sample. This might be a limiting factor for the interpretation of the

results. Nonetheless, as expected there were significant differences in diet composition between groups. Those with reduced CHO intake consumed significantly more meat (white, red and all types of processed meats) and butter but perhaps surprisingly also more vegetables. They also consumed significantly less fats and oils, legumes, fruit and soft drinks. After adjusting for socio-economic status only red meat consumption remained significantly different. That those with reduced CHO intake obtained 14.8%TE from SFA and 16.8%TE led the author to caution against the perceived unhealthiness of this diet. In addition, there was a concern regarding some micronutrients (magnesium, potassium and selenium) and fibre intake. These were below recommendations in both groups. The intake of red meat, butter and processed meats and their potential detrimental effects were also highlighted. However, although the study reported blood lipid and fasting blood glucose concentrations (LDL-C, HDL-C and GLUC were significantly higher in the reduced CHO group) these had only been obtained from less than half the sample limiting applicability of the findings. The concerns by the author were based on general concerns surrounding LC diets but the analysis was not of an inferential nature regarding the impact of reduced CHO intake on CM health. As the present study showed similar intake values for the lowest quartiles of CHO and the highest quartiles of SFA intake respectively, it can fill this gap and can address the concerns regarding the impact of reduced CHO, high-SFA diets, which actually seem to be positive. In this respect, the study by Guess and the present study can complement each other.

The National Health and Nutrition Examination Survey (NHANES) dataset is representative of the US population similar to the NDNS in the UK. The majority of participants are of white Caucasian ethnicity and findings from NHANES analyses might therefore have a degree of applicability to the findings from the present study. Zhu et al.. (2004) analysed the data from 11,239 individuals from the 1988-1994 NHANES survey waves and ran unadjusted and demographic and lifestyle adjusted models to investigate the impact of low (<40%TE), moderate (40-60%TE) and high (>60%TE) total CHO intake and of low (<30%TE), moderate (30-40%TE) and high (>40%TE) total fat intake on the odds of MetS. There were significant findings for the total male sample and normal-weight and overweight males with moderate and/or low CHO intake decreasing the odds of MetS in both unadjusted and adjusted models. In both the unadjusted and adjusted models high fat intake significantly decreased the odds of MetS for both men and women. Although the authors did not investigate the impact of subtypes of CHO or fatty acids their findings somewhat mirror the ones from the present analysis of the NDNS. Interestingly, the distribution of odds was also not necessarily linear, meaning that in some cases the odds of MetS were lower with

moderate intake than with low CHO intake. Whilst the present analysis utilised natural quartiles as derived from the sample, Zhu et al. (2004) used cut-off points reflecting US dietary guidelines of the time. The number of subjects per intake category was not stated. Furthermore, moderate dietary intake covered a wide range through which subjects consuming either 40%TE of CHO or 60%TE of CHO would both be classed as moderate consumers. The use of natural quartiles in the present study resulted in narrower ranges in the second and third quartile making it easier to attribute potential CM benefits of dietary intake to more precise values. Whilst Zhu et al.'s (2004) findings mean that potentially deriving less than 60%TE from CHO might be preventative against CM risk, the present analysis shows that in the majority of the separate analyses MetS was avoided with CHO intakes below 41%TE. In addition, as the quartiles of dietary intake created were also stratified by gender, BMI group and age group this can potentially reflect how dietary intake might affect individuals differently based on their gender, age or BMI. In future, this might make it possible to make more personalised recommendations to use diet as a successful tool to prevent or ameliorate CM risk.

Logistic regression analysis of data collected from 3,324 Americans in the 2007 – 2012 NHANES survey waves (Ha et al., 2018) found no significant association between CHO intake and MetS prevalence unlike the present analysis of the NDNS. However, for both men and women there was a significant trend of reduced HDL-C concentrations with increasing CHO intake (Ha et al., 2018). For women there was also a significant non-linear positive association between CHO intake and fasting blood glucose concentrations and a significant linear positive association between CHO intake and TG concentrations. Data was analysed by quintile allowing for assessment of differential effects of high intakes of CHO (Median 64.6%TE for men and 66.9%TE for women). Models were adjusted for demographic and lifestyle factors but not total energy intake, which is common practice in nutritional epidemiology to allow for potentially confounding effects of high or low caloric intake (Bates et al., 2019; Rhee et al., 2014; Willett et al., 1997) and is therefore potentially a limiting factors for the applicability and comparability of the findings.

Mazidi et al. (2017) more recently undertook some work to identify potential associations between dietary intake and MetS and its components. Using Principal Component Analysis (PCA), they identified three distinct dietary patterns in the 2001-2012 datasets of the National Health and Nutrition Examination Survey (NHANES) including 23,157 participants (6561 with MetS). One of these patterns was mainly representative of SFA and MUFA intake and to a lesser degree of CHO intake. After adjusting for age, gender and

ethnicity trends showed that there was an increase in the intake of each of these nutrients across quartiles, so that those in the highest quartile of this pattern consumed the most of each SFA, MUFA and CHO and those in the lowest quartile the least of each. However, rather than basing dietary intake on %TE quartiles were assessed in g/d, which means that even in the pattern coined SFA-MUFA CHO intake was eight times higher than SFA intake and of MUFA intake. The daily amount of SFA and MUFA consumed was nearly equal. The emphasis on the effects of SFA and MUFA seems therefore perhaps somewhat misleading. This dietary pattern was associated with significantly increased overall odds of MetS and of high WC and high TG in the two highest quartiles of intake. Further analysis did not show any associations between individual macronutrients and MetS. Although the researchers adjusted for a number of confounding factors of CM risk, they also did not adjust for total energy intake, potentially a limiting factor (see above). In the present analysis of the NDNS the potential synergy of macronutrients was not considered but focus was placed on individual macronutrients. Whilst there were significant findings for individual macronutrients, future analysis of dietary patterns might show synergistic effects that can be important for day-to-day practical dietary intake.

Jin and Nicodemus-Johnson (2018) applied a similar approach to Mazidi et al. (2017) by deducing three distinct dietary patterns based on the data collected from 12,284 participants from the 2001-2013 NHANES survey waves. Primary outcomes in this study were individual components of CM risk rather than MetS. The researchers undertaking this secondary analysis did adjust for total energy intake in addition to other lifestyle and demographic factors. Their patterns were similar to those of the previous study, of which one was positively correlated with SFA and MUFA and negatively with CHO, fibre and folate. Another pattern was positively correlated with MUFA and PUFA consumption and negatively with SFA, CHO, sugar and calcium intake. The authors then also split the data into quartiles of dietary intake as g/d rather than %TE. However, whilst in the first pattern CHO intake decreased across quartiles and SFA and MUFA intake increased, overall CHO intake was still nearly seven times higher than SFA and MUFA intake each. In the second pattern, CHO and SFA intake decreased across quartiles and MUFA and PUFA intake increased, but nonetheless CHO intake was still seven times higher than MUFA intake and over ten times higher than PUFA intake. It is therefore not clear whether overall CHO and fatty acids intake would have been classed as low, moderate or high. For the first dietary pattern, lowest CHO intake and highest SFA/MUFA intake resulted in significantly lower HDL-C concentrations in men, hence potentially increasing the risk of MetS depending on the levels of other MetS components. For the second pattern, lowest CHO/SFA intake and

highest MUFA/PUFA intake resulted in significantly higher HDL-C concentrations and significantly lower TG concentrations in men and significantly lower TG concentrations in women. This would potentially decrease the risk of MetS and is somewhat in line with the findings in the present study of beneficial effects of lower CHO intake but contradictory regarding the beneficial effects of higher SFA intake. When examining individual nutrients Jin and Nicodemus-Johnson (2018) found significant inverse associations between total CHO and HDL-C concentrations and sugar and HDL-C concentrations and between total fat and TG concentrations in men. In women, there were significant inverse associations between total CHO and HDL-C concentrations and sugar and HDL-C concentrations and significant positive associations between total CHO and TG concentrations. Although the analysis did not consider MetS as a whole but only individual components of it, the findings are nevertheless of interest for the present analysis. Elevated TG concentrations and reduced HDL-C concentrations are amongst the hallmarks of the MetS diagnosis and they worsened with total CHO or sugar intake. The findings from the present analysis of increasing odds of MetS with higher CHO intake are in line with the literature. Jin and Nicodemus-Johnson found no significant associations between SFA intake and individual MetS components, which might either point to a neutral effect of SFA in general, or the intake of SFA was too low to have an effect. However, it has to be noted that whilst assessed as an individual nutrient high CHO intake was potentially detrimental to CM health, once assessed as component of a dietary pattern the associations were less clear. This must lead to the conclusion that overall diet quality might have been a confounding factor in the analysis that was not considered.

Praagman et al. (2016) used data from 35,597 participants of the Dutch cohort of the European Prospective Investigation of Cancer (EPIC) recruited between 1993 and 1997 to examine associations between fatty acid intake and ischemic heart disease (IHD). Data was collected via FFQ and analysed in several step-wise adjusted models which included demographic and lifestyle data and aspects of dietary intake. In the final adjusted model short- and medium-chain SFAs and odd-chain SFAs were inversely associated with IHD. Praagman et al. (2019) also analysed data collected 53,375 participants from the Danish EPIC study. This showed that in adjusted models there was a significant non-linear inverse association between SFA intake and risk of myocardial infarction (MI). Those in higher intake quartiles were at lower risk. These differences persisted for the individual SFAs lauric and myristic acid. Pooled intakes of butyric, caproic, caprylic and capric acid were also significantly non-linearly inversely associated with MI risk. Whilst intakes of palmitic and stearic acid per se were not significantly associated with increased MI risk nonetheless

replacing them with plant protein significantly reduced MI risk. Whilst the present study is unable to provide details on the intake of individual SFAs its non-linear inverse association of SFA intake with MetS odds is in line with findings by Praagman et al. for total SFA. Whilst Praagman et al. assessed outcomes different from MetS all three studies dealt with CM risk outcomes. However, whilst the NDNS dietary intake and CM risk data are collected very closely together (within about two to three months from each other (Bates et al., 2016)), the time difference between dietary collection and collection of outcome data was on average 12.2 years in the Dutch and 13.6 years in the Danish cohort, during which dietary habits might have changed, potentially limiting the validity of the findings.

The sometimes conflicting and non-linear findings regarding dietary intake of carbohydrates and fats and MetS also highlight that ideally, diet should not be assessed from a purely quantitative perspective but that diet quality, for example derived from the intake of sub-types of individual fatty acids, plays an important role. Whilst the NDNS datasets provide details on the intake of mono- and disaccharides, the same detail is lacking for the composition of foods containing fatty acids and only data for SFA, MUFA and PUFA is provided. As the present analysis shows that there might be more differential effects of SFA, in future it would be advantageous if the organisations undertaking the initial analysis of the NDNS food diaries would provide further detail on sub-categories of the individual fatty acids consumed by the UK population similar to data provided in the NHANES datasets (Mazidi et al., 2017).

### 2.5 Study limitations and future work

There are a number of limitations associated with national nutritional surveys in general and the NDNS RP in particular. Firstly, self-reported methods are known to have limitations due to reporting bias caused by the desirability or socially unacceptability of some food items (Desroches et al., 2013; Maurer et al., 2006). Furthermore, specific populations in particular have been shown to underreport dietary intake, such as women, the elderly and the obese (Castro-Quezada et al., 2015; Maurer et al., 2006). Three and four-day food diaries (as used in the NDNS RP) have the advantage of being more immediate, especially if participants are instructed to record foods and drinks as and when they consume them, so that memory-bias is less likely. Nonetheless, being conscious of taking part in a dietary study participants might have either changed their consumption habits subconsciously on those days due to desirability/undesirability of certain items or due to the burden of having to record everything they ate. Although the limitations of the dietary intake collection tool used have to be born in mind, this present study followed the recommendations made by Subar et al.

(2015) who advised to use self-reported dietary intake as an adjustment tool for intake of other self-reported nutrients. This is what the present study set out to do and no inferences were made between energy intake and CM risk status.

Another potential limitation is the time gap between collection of the dietary and the blood marker data. The food diaries are completed during the first stage of data collection, the blood samples are drawn during stage 2 with about two to three months interval between these (Bates et al., 2016). Further details on the time schedule of the survey waves and two stages can be found in Appendix B to the NDNS reports (Tipping, n.d.). Depending on the half-life of surrogate endpoints of health and disease status, such as for example HDL (Kuai et al., 2016; Yetley et al., 2017), these might no longer accurately reflect the self-reported dietary intake. In addition, concentrations might display seasonal variability (Robinson et al., 1992; Shahar et al., 1999) and could be higher than normal if the blood sample was taken not long after a period of increased or adjusted dietary intake, such as the Christmas season (Vedel-Krogh et al., 2019). Future analysis of the NDNS dataset can take this into account by using the month when the blood sample was drawn as a potential covariate.

Although the multi-stage stratified and clustered sample design of the NDNS RP and the use of boost samples from the devolved nations give it the potential to be representative of the UK population in practice this might lead to underrepresentation of specific groups that are more likely to decline participation. It also contains the danger that high response rates from some clusters result in overrepresentation of these. Whilst this is generally acknowledged in national surveys, such as the NDNS RP and NHANES, and addressed through the use of weights it can lead to inaccurate findings in the analysis. In particular, the logistic regression has shown a few extremely high or extremely low values and wide CIs. Whilst some of this might be addressed by using a simpler model, in some cases it might mean that the sample is not representative of the UK population. It is therefore most advantageous that the NDNS in its current format continues to collect data on an annual basis to add to the evidence base. With the growth in sample size future analyses will most likely gain in accuracy and precision. This will make extrapolation of the results to the general population also more robust and valid (Ferrill et al., 2010).

Whilst the NDNS RP is a valuable and useful tool to examine correlations between diet, lifestyle and CM risk, in future it can be useful to measure additional biochemical markers, such as apolipoprotein A-1, apolipoprotein B and small dense LDL-C to enable researchers to make more informed and differential assessments of CM health and disease in the UK population. Furthermore, like its counterparts in the US and Korea the NDNS RP would

benefit from more sophisticated assessments of body composition, such as dual-energy x-ray absorptiometry (DXA) (Hinton et al., 2017; Kim et al., 2015). This would allow for correlations between dietary intake, diet quality and location and distribution of adipose tissue, a known risk marker of CMD (Tchernof and Després, 2013).

As discussed in chapter 2.4 future dietary intake data should include subtypes of fatty acids to permit more refined and differential analysis of the impact of these on CM health and disease. More evidence is emerging of the distinct effects of short and medium-chain, long-chain and branched chain fatty acids on blood lipids (Iggman and Risérus, 2011) and future analysis of dietary intake data of the UK population would benefit from incorporating these data. This would make future dietary recommendations more effective.

In the meantime further work to build on findings from the present study should include an analysis of dietary patterns (DP) and CM risk to compare these with research previously undertaken on the NHANES survey waves (see chapter 2.4). Roberts et al. (2018) most recently used the NDNS 2008-2012 RP data from 2083 adults to derive four DPs using Principal Component Analysis. Correlation between DPs ('Snacks, fast food, fizzy drinks' (SFFFD), 'Fruit, vegetables, oily fish' (FVOF), 'Meat, potatoes, beer' (MPB,) and 'Sugary foods, dairy' (SFD)), demographics, nutrient intake and diet quality were assessed. The majority of patterns were correlated with specific genders, age groups, smoking status and socio-economic status and with varying intakes of micro and macronutrients. The FVOF DP was positively associated with higher diet quality and the SFFFD and MPB negatively associated with it. However, this research focused solely of demographic and nutrient intake and therefore analysis of DP of the UK populations and the correlation with CM risk is urgently needed.

The analysis undertaken for this study used the complete white adult sample for which presence of MetS could be established. As the majority of findings showed that higher intake of CHO or lower intake of SFA presented with higher odds of MetS, it would be valuable to explore the dietary characteristics of these specific quartiles in more depth. This could be done by further dividing these individual quartiles into subcategories of intake.

The present study focused on the association between stratified intake of dietary carbohydrates and fats on MetS as a cluster of CM risk factors. Next steps should include examining correlations between individual components of MetS risk to assess whether there are differential impacts of these macronutrients on these separate risk marker in stratified analyses. Furthermore, the NDNS RP can be used to contribute to the debate surrounding

SFA and LDL-C, which should also investigate whether there are food group-specific associations.

### 2.6 Conclusion

The novelty of the present analysis is that it is the first to examine the associations between dietary intake and CM risk and MetS in particular using the appropriate statistical method of complex sample analysis. However, due to the nature of the NDNS RP as cross-sectional study correlation does not mean causation (Zhu et al., 2004) and it is therefore necessary to investigate the links between diet and CM risk in an intervention study. Contrary to current dietary advice, across sex, age and BMI stratifications those with reduced total CHO intake or reduced intake of either total starches or total sugars and those with increased SFA intake were at lower odds of presenting with MetS. These results were most consistent when data was stratified by age group. Further studies are needed to explore the associations in more depth, including the potential contribution that individual foods groups and subgroups of macronutrients, specifically fatty acids, make. This also includes the application of dietary pattern analysis.

Based on the findings from the present analysis that lower CHO intake and higher SFA intake is associated with decreased odds of MetS it was decided to delve deeper into this topic by designing a study that had the potential to observe the effects of very high reductions of CHO intake and potentially very high increases in SFA intake. The aim of this was to investigate differential effects and possible cause-effect relationships between diet and CM risk markers in a randomized sample.

## Chapter 3 – Study 2: The CALIBER study

### 3.1 Introduction

Analysis of the NDNS dataset with focus on selected surrogate endpoints of CM risk showed that there were several significant findings from a number of stratified multi-variate models regarding the intake of CHO and SFA. It were mainly those in higher quartiles of CHO intake or lower quartiles of SFA intake who showed increased odds of presenting with MetS. The next step was therefore to design and implement an intervention study that built on these findings and exposed a sample population to habitual lower intakes of CHO (<50g/d) and habitual higher intakes of SFA (assumed to occur with total dietary fat intake ≥65%TE). The diet that is characterised by this combination and that hence allows for a more targeted investigation into the effects of lesser CHO and higher SFA intake is the so-called "low-carbohydrate, high-fat diet" (LCHF). Although this diet is not specifically aiming at high consumption of SFA, an increased intake of SFA is a natural result of higher intakes of meats and dairy products, which tend to substitute CHO-rich foods (Tay et al., 2008; Volek et al., 2004; Wylie-Rossett et al., 2013).

To build on the findings from the secondary analysis of the NDNS RP 2008-2014 date the second study assessed the impact of prescribed CHO and fat intakes on a number of factors associated with CM risk, including clinical markers, body composition and physical activity levels. This included a number of markers that have not been investigated in great depth in LCHF diet thus far, such as the leptin/adiponection ratio and fibroblast growth factor 21. In this context other factors affecting the potential effectiveness of diets were also investigated, namely the adherence to the prescribed diets and the experience of food cravings by the participants.

#### 3.1.1 Dietary intake, adherence and food cravings

As outlined in chapter 1.0 there is continuing debate on whether current dietary guidelines, including the UK EWG, are correct in recommending CHO intakes of ≥50%TE per day. It is important to note that different categories of diets have been developed depending on amount of carbohydrates consumed. Diets have been categorised as very low-carbohydrate or ketogenic (VLC or KD), low-carbohydrate (LC), moderate-carbohydrate (MC) and high-carbohydrate diets (HC) with decreasing levels of carbohydrate restriction (Figure 3.1).


Figure 3.1 – Categorisation of diets based on amount of carbohydrates consumed

Categorisation based on Accurso et al., 2008; Naude et al. (2014) further differentiate between a 'balanced' and 'high' CHO diet (45-65% and >65% energy respectively); the RDA has been defined as "the average daily dietary intake level sufficient to meet the nutrient requirements of nearly all (97–98 percent) healthy individuals in a group" (Otten et al., 2006, p.102)

The decreasing amounts of carbohydrates (CHO) are either replaced by increasing the protein or the fat content of the diet or a combination of both (Naude et al., 2014; Seidelmann et al., 2018). Diets focusing on increased fat intake in this context are known as LCHF diets (Brouns, 2018). However, there is no overall consensus regarding the definition of LC with a number of systematic reviews and meta-analyses including studies with CHO intake ranging from anywhere between 4% energy up to 45% energy (Churuangsuk et al., 2018; Gjuladin-Hellon et al., 2018; Hu et al., 2014; Huntriss et al., 2018; Johnston et al., 2014; Naude et al., 2014; Seidelmann et al., 2018).

Ketogenic diets serve the purpose of inducing nutritional ketosis in most humans, at which point ketone bodies are produced, mainly derived from fats. Ketones are used as fuel in place of CHO and generally produced with a CHO intake of <50 g/d, but this can slightly differ between individuals (Harvey et al., 2018). Fat intake during a ketogenic diet amounts to ≥65%TE (Barzegar et al., 2019).

Adherence is important in any dietary or other health-related intervention and has been defined as "persistence in the practice and maintenance of desired health behaviors and is the result of active participation and agreement" (Cohen, 2009, 27). Food cravings, defined as "an intense desire to consume a specific food", amongst other factors have been found to impact adherence to dietary prescriptions (Richard et al., 2017).

### 3.1.2 Clinical markers of cardiometabolic risk

#### 3.1.2.1 Surrogate markers that are secondary targets in primary and secondary CVD prevention

National guidelines for the prevention and management of CV risk inform health care professionals' decisions on which markers to focus on in their practice. Whilst reducing concentrations of LDL-C remains a primary target in a number of countries (Catapano et al., 2016; Grundy et al., 2018), the usefulness of additional markers to aid assessment of CVD risk is being recognised. These include apolipoproteins (Grundy et al., 2018) and plasma lipid ratios (Catapano et al., 2016).

##### 3.1.2.1.1 Apolipoproteins

Apolipoproteins are major structural components of plasma lipoproteins and determine their overall function. A range of apolipoprotein classes and subclasses has been identified (Mahley et al., 1984) and in recent years they have been noted as potential surrogate markers of increased CM risk (Arsenault et al., 2011). Two apolipoproteins were of interest in the CALIBER study, apolipoprotein A-1 (apoA1) and apolipoprotein B (apoB).

Apolipoprotein A-1 is a major structural component of HDL making up about 70% of the HDL particle. One of its main functions is reverse cholesterol transport. It is therefore instrumental in providing HDL with its anti-atherogenic ability to remove cholesterol particles from the blood circulation (Mangaraj et al., 2016). Apolipoprotein A-1 levels are inversely associated with CMD and seem to remain so even with the increasing risk of very high HDL-C levels (van der Steeg et al., 2008). Women seem to present with higher apoA1 concentrations than men (Marti et al., 1989; Sniderman et al., 2016); concentrations are also positively correlated with increasing age (Sniderman et al., 2016; Zhao et al., 2018). In adult women reference ranges vary from 101 mg/dL to 224 mg/dL; in adult men the reference ranges are between 95 mg/dL and 186 mg/dL (Gloucestershire Hospitals Foundation Trust , 2017).

Higher total CHO intake has been associated with decreases in apoA1 concentrations (Mensink et al., 2003; Mente et al., 2017). Mensink (2016) calculated that replacing CHO with SFA was associated with an increase in apoA1 levels, whereas replacing SFA with CHO had the opposite effect. Total fat intake and consumption of subtypes of fats have also been associated with higher apoA1 concentrations (Mente et al., 2017). Tognon et al. (2012) analysed the data from 2,907 adults who took part in the cross-sectional Swedish INTERGENE study. They found that for women for each 10g/d increase of SFA apoA1 concentrations significantly increased by 1.3%. It could be argued that in the context of

CMD prevention higher CHO intake therefore increases CM risk. However, as there are still many outstanding questions to solve regarding the significance of apoA1 concentrations and the scale of the impact of CHO intake this remains speculation. One important issue to bear in mind in this context is the quality of the CHO-rich foods consumed. Frondelius et al. (2017) analysed the FFQs from a cross-sectional study of 24,984 Swedish adults. They found that it was sucrose and foods containing added sugars (i.e. low-quality CHO) that were associated with lower apoA1 concentrations. It appears that not all SFA have the same effect on apoA1 concentrations and that the food source and accompanying food matrix might play a role. In the cross-sectional analysis (see above) by Frondelius et al. (2017) intake of fermented milk and cheese was positively associated with apoA1 concentrations. More recently Panth et al. (2018) undertook a systematic review and meta-analysis of 11 cross-over and one parallel trials with a total of 299 participants to examine the impacts of medium-chain saturated fatty acids (MCSFA) (contained in coconut or palm kernel oil) and long-chain saturated fatty acids (LCSFA) (contained in dairy, tallow, palm oil and lard) on blood lipids. Two of the studies (with 74 subjects) were in hypercholesteraemic participants, the rest were healthy adults. The evidence whether there are differential responses between the healthy and those at increased CM risk is therefore limited. Medium-chain SFA significantly increase apoA1 concentrations compared to LCSFAs. There is scarcity of LC diets examining the impact of CHO reduction on apoA1 concentrations. Volek et al. (2009) investigated the impact of a hypocaloric LCHF diet on apoA1 concentrations in 20 overweight or obese adults with elevated TG and reduced HDL-C concentrations over 12 weeks. Apolipoprotein A-1 concentrations increased non-significantly during this time.

Apolipoprotein B is the main apolipoprotein of LDL and other triglyceride-rich lipoproteins. In the majority of fasted individuals, about 90% of circulating apoB are contained within LDL particles (Carr et al., 2019). Women seem to present with lower apoB concentrations than men (Marti et al., 1989; Sniderman et al., 2016); concentrations are also inversely correlated with increasing age (Sniderman et al., 2016). Apolipoprotein B has been found to be an equal if not superior risk predictor of CMD risk to LDL-C (Carr et al., 2019; Grundy et al., 2018), although this remains debatable and has not reached consensus in many countries. Currently in Europe apoB is a secondary treatment target in CVD treatment and prevention (Catapano et al., 2016). The most recent American Heart Association Guidelines on the Management of blood cholesterol (Grundy et al., 2018) and American College of Cardiology/American Heart Association Guideline on the Primary Prevention of Cardiovascular Disease (Arnett et al., 2019) included apoB concentrations ≥130 mg/dL as risk enhancing factor in adults without T2DM (aged 40-75 years) at 10-year intermediate

risk of CVD. Grundy et al. (2018) also advise to measure apoB to check whether confirmed hypertriglyceridaemia is atherogenic. The 2018 update to the 2016 Canadian Cardiovascular Harmonized National Guidelines Endeavour (C-CHANGE) guideline for the prevention and management of cardiovascular disease in primary care (Tobe et al., 2018) re-iterates the 2016 recommendations by Anderson et al. (2016) to use apoB as an alternative target variable to LDL-C in dyslipidaemia (apoB <0.8g/L).

Higher CHO intake has been associated with decreased apoB concentrations (Mente et al., 2017), which confirmed calculations by Mensink (2016) that replacing SFA with CHO would decrease apoB concentrations. Mensink (2016) also found that replacing CHO with SFA would increase apoB concentrations. The association of apoB with TG-rich (atherogenic) lipoproteins would therefore strongly suggest that lowering CHO intake and increasing SFA intake (i.e. following a LCHF diet) would increase CM risk.

Similar to apoA1 the quality of the CHO-rich foods consumed has to be taken into account. Frondelius et al. (2017; see above) found that sucrose and foods containing added sugars (i.e. low-quality CHO) were associated with higher apoB concentrations. One study compared the effects of SFA-rich foods. Thorning et al. (2015) found no changes in apoB concentrations with a cheese (96g/d – 120g/d depending on overall energy intake) or a high-meat diet (up to 164g/d) in their 10-week randomised-cross-over study in 19 postmenopausal women. The diets had been designed to provide 15%TE from protein, 35%TE from fat and 50%TE from CHO. Only a few LCHF trials have investigated the impact of adhering to the diet on apoB concentrations. These included trials by Tay et al. (2008), Brinkworth et al. (2009) and Volek et al. (2009). Tay et al. (2008) found that in 45 abdominally obese adults on a hypocaloric LCHF diet apoB concentrations had not changed after six months. Thirty-three adults with abdominal obesity and at least one additional CM risk factors completed a one-year RCT conducted by Brinkworth et al. (2009). They were asked to follow a hypocaloric LC diet. After this time, apoB concentrations had non-significantly increased. In contrast to the findings made by Brinkworth et al. (2009) and Tay et al. (2008), Volek et al. (2009) found that in the 20 overweight or obese adults with elevated TG and reduced HDL-C on a hypocaloric LC diet apoB concentrations had non-significantly decreased after 12 weeks. It can only be speculated whether the amount of time spent on the intervention might have been a confounding factor for these findings. The 3-months decrease observed by Volek et al. (2009) could potentially level out after six months (similar to Tay et al. (2009)) and possibly increase further after 12 months like in Brinkworth et al. (2009). More

long-term studies of LCHF diets should therefore in future examine the impact on apoB concentrations to reflect that this marker can add value to the prediction of CVD risk.

#### 3.1.2.1.2 Plasma lipid ratios

In addition to the TG/HDL-C ratio other lipid ratios have also demonstrated to be potentially good if not superior predictors of increased CM risk. One of these is the apolipoproteinB/apolipoproteinA-1 ratio (apoB/apoA1) The apoB/apoA1 ratio has been found to be the best predictor of CVD risk in a 10-year follow-up study of 2,583 Greek men and women (Nomikos et al., 2015). It was also one of the strongest risk factors and of myocardial infarction (MI) in the INTERHEART study, which included data from 52 countries. The odds of MI were significantly linearly and positively associated with increasing apoB/apoA1 ratio (Yusuf et al., 2004). However, other analyses have found that the apoB/apoA1 ratio did not add additional benefits to CVD risk prediction. Van der Steeg et al. (2007) reported that it actually misclassified 41.1% of cases (n=869) and 50.4% of controls (n=1511) aged 45 – 79 years in the EPIC-Norfolk cohort when data was used to develop models for coronary artery disease prediction. Target levels in primary prevention of CVD have been set at <0.9 for men and <0.8 for women. Target ranges in secondary prevention of CVD have been set at <0.7 for men and <0.6 for women (Millán et al., 2009). One current limitation is that the majority of inferences appear to have been drawn from cross-sectional and observational studies. There has been a scarcity of RCTs investigating the validity of the apoB/apoA1 ratio.

Higher total CHO intake and higher sucrose intake in particular has been associated with higher TG/HDL (see chapter 2) and apoB/apoA1 ratios (Mente et al., 2017; Tognon et al., 2012b). This means that higher CHO consumption might be detrimental to CM health and increase risk of CMD. When SFA was replaced with CHO the impact on the TG/HDL ratio was detrimental and vice versa (Mensink, 2016). As for apoA1 and apoB assessed in isolation, the quality of CHO and the food source of SFA appears to play an important role. Frondelius et al. (2017) (see above) also found that sucrose and foods containing added sugars showed a positive association of apoB/apoA1 ratio. Fermented milks and cheese were negatively associated with apoB/apoA1 ratio. Volek et al. (2009) reported no significant changes in apoB/ApoA1 ratio after 20 overweight and obese adults followed a KD for 12 weeks. The type of SFA consumed (Mensink, 2016) seems to have a differential effect on serum lipid and lipoprotein ratios with replacement of CHO with specific SFA, namely lauric, myristic or palmitic acid, leading to decreased TG/HDL ratio. A number of LCHF studies have also reported the effect of dietary intake on TG/HDL ratio and consistently

found significant improvements regardless of study duration or sample population (Brinkworth et al., 2009; Meckling et al., 2002; Meckling et al., 2004; Sharman et al., 2004; Urbain et al., 2017; Westman et al., 2002; Volek et al., 2004; Volek et al., 2009; Yancy et al., 2004). The majority of studies were RCTs that compared a hypocaloric or an ad libitum LC diet with a low-fat diet. Most recently, Harvey et al. (2018) reported on a trial randomising 39 overweight and obese healthy adults to one of three CHO-restricted diets for 12 weeks. Reported CHO intakes were 22.5%E, 14.1%E and 7.9%E. The TG/HDL ratio decreased significantly in all three diet arms but the decrease was greatest in the group consuming the least amount of CHO.

#### 3.1.2.2 Small dense lipoprotein cholesterol

As already discussed, reduction in LDL-C levels remains the primary target for CVD prevention in many CVD guidelines (Catapano et al., 2016; Grundy et al., 2018). However, over the past couple of decades this purely quantitative approach has come into question and the particle number, size and functionality of these lipoproteins has been the focus of intense investigation (Diffenderfer and Schaefer, 2014; Kontush, 2015; Namiri-Kalantari et al., 2015). There seems to be more evidence that the size of specific lipoprotein particles and their numbers are superior markers of CMD risk compared to overall cholesterol concentrations (Mora et al., 2013; Otvos et al., 2011; Sniderman et al., 2015). Higher small dense LDL cholesterol (sdLDL-C) concentrations been associated with a more atherogenic pattern of CVD (Diffenderfer and Schaefer, 2014; Ivanova et al., 2017). Those presenting with large buoyant LDL cholesterol (lbLDL) as the dominant LDL subtype are categorised as pattern A, whereas those presenting predominantly with sdLDL as pattern B (Austin et al., 1988; Austin et al., 1990; Krauss, 2010). The latter is more atherogenic and hence those falling into the pattern B category as classed as being at higher risk of CMD. The combination of low HDL-C, high triglycerides and sdLDL is known as atherogenic dyslipidaemia and extremely detrimental to CM health (Siri-Tarino et al., 2015). However, it can be argued that elevated LDL particle number is the greater predictor of CV risk but epidemiological evidence on high LDL-P number from lbLDL and diminished sdLDL, and intermediate LDL is lacking.

Consuming refined CHO to reduce SFA intake seems to raise sdLDL and consequently increase CM risk (Siri-Tarino et al., 2010), whereas KDs have been shown to exert beneficial effects on sdLDL patterns and to reduce sdLDL particle numbers (Noakes and Windt, 2017). In the parallel cross-over study conducted by Sharman et al. (2004) 75% of the overweight and obese men who had presented with predominantly sdLDL patterns at baseline decreased

their numbers of sdLDL particles on a six-week hypocaloric LCHF diet. As the primary outcome of this particular study was weight loss the two experimental diets (the other a LF diet, <25%TE from fat) were not separated by a washout period, which might have had confounding effects on the results. A four-week study with 10 normal-weight women (Volek et al., 2003) reported significantly increased LDL-C concentrations at endpoint but concentrations of LDL subclasses did not change. Three of the women showed moderate to large increases in peak LDL size, moving away from the pattern B (see above). In a subsequent study, 13 overweight healthy women followed a hypocaloric diet for four weeks (Volek et al., 2004). Neither LDL-C nor percentage of LDL subclasses changed significantly. When prescribing a hypocaloric 12-week KD diet to 20 overweight or obese adults at increased CM risk Volek and co-workers (2009) reported that, although LDL-C levels did not change much, there was a shift to less atherogenic patterns. The majority of the LCHF studies reporting on sdLDL-C were hypocaloric and not ad libitum. It remained to be seen whether the beneficial effects on sdLDL-C would still be observed without prescribing an energy deficit.

#### 3.1.2.3 Insulin concentrations and insulin resistance status

The hormone insulin is produced and released by the human body in response to circulating glucose and amino acids. One of its roles is to facilitate the transfer of glucose into muscle and adipose tissue (Wilcox, 2005). It also acutely stimulates lipogenesis whilst downregulating lipolysis in adipose tissues (AT) (Ludwig, 2018). Insulin resistance increases in the presence of increased visceral adipose tissue (Tchernof and Després, 2013) and often marks the beginnings of T2DM, presents with other (cardio)metabolic disturbances. It is a state in which the insulin pathway is downregulated and insulin receptors in skeletal and adipose tissues become less effective in facilitating intra-cellular transport of glucose into the tissues (Boucher et al, 2014: Shanik et al., 2008). The circulating glucose can longer be effectively taken out of the system leading to hyperglycaemia (Yaribeygi and Sahebkar, 2018). Insulin resistance has been measured by a number of tools, which have been reviewed by Antuna-Puente et al. (2011). The gold standard measure is the hyperinsulinaemic–euglycaemic clamp test, which is time consuming and invasive and can therefore be prohibitive to use in some studies. Other less invasive and easier to implement methods include the Quantitative insulin-sensitivity check index (Quicki) and the Homeostasis Model Assessment (HOMA), which calculate IR from fasting insulin and fasting blood glucose concentrations. Originally proposed in 1985 (Matthews et al., 1985), the latter has since been updated to HOMA2 to allow for other physiological/endocrinological factors to be taken into consideration, allowing for a more

dynamic rather an a linear approximation of IR (Levy, 1998). The developers of HOMA2 at the University of Oxford Diabetes Trials Unit therefore recommend that the freely available HOMA2 calculator is used to calculate IR (see chapter 3.4.6) (Levy, 1998).

The quality of CHO consumed plays an important role in how IR status is affected and whilst it has been claimed that HC diets are more beneficial for insulin sensitivity, the author conceded that this might be in the context of a low-glycaemic as opposed to a high-glycaemic index diet (Wilcox, 2005). Schwingshackl et al. (2018) concluded in their systematic review and network meta-analysis of 66 trials (3595 normal-weight, overweight or obese adults; 280 adults with T2DM) that wholegrains, refined grains and to a lesser degree fruit and vegetables are amongst the best food groups to reduce IR. Malik and Hu (2015) found that sugar-sweetened beverages might have detrimental effects on glycaemic control and increased IR. However, in smaller amounts and in exchange for other CHO in the diet fructose seems to exert a positive effect on glycaemic control. Only when fructose is consumed as additional energy an increase in IR seems to occur (Khan and Sievenpiper, 2016).

Positive associations between SFA consumption and hyperinsulinemia have been reported (Siri-Tarino et al., 2010; Wilcox, 2005). However, it has been highlighted that the effect of SFA might be dependent on the amount of total dietary fat consumed. In the context of lower-fat diets the proportion of SFA consumed might have an overall detrimental effect on CM health than in higher-fat diets (Siri-Tarino, 2010). A systematic review of 10 weight stable RCTs (cross-over and parallel) in normal-weight, overweight and obese healthy adults (and one small study in children) conducted by Turner et al. (2015) found that intake of high-fat dairy had mainly neutral or positive effects on insulin sensitivity. However, study duration played a part in this with trials lasting less than two months finding no effect, whereas interventions with a duration of between three and six months found mainly improvements. Turner et al. (2015) conducted a cross-over RCT in which a sample of 47 overweight or obese adults with or without impaired fasting glucose or impaired glucose tolerance were allocated to a lean red meat SFA or a low-fat dairy SFA diet for four weeks each. In the overall sample, and in women, IR was significantly greater after the dairy diet than with the red meat diet but the findings for the male sample alone were not significant.

A decrease in fasting plasma insulin concentrations is a common outcome in LCHF trials. Santos and colleagues (2012) conducted a systematic review and meta-analysis that included reports on 13 RCTs with a total of 1141 obese participants. Each trial had ≥100 completers. They found that after a LCHF diet there was a significant decrease in insulin levels of 2.24

μIU/mL. In the study conducted by Harvey et al. (2018) (see above) the group consuming the least amount of CHO presented with the largest reductions in insulin concentrations, although all three LC diets showed beneficial effects on insulin levels. Some The LCHF studies in which significant decreases in IR were observed in male (Veum et al., 2017), female (Volek et al., 2004) and mixed populations (Volek et al., 2009) were of short duration and lasted for a maximum of 12 weeks. In another short study Sharman et al., (2002) found no effects on IR status in men after 3 or 6 weeks on a LCHF diet. It therefore unclear at this point what the long-term effects of a LCHF diet would be on IR.

#### 3.1.2.4 Adipokines and hepatokines

##### 3.1.2.4.1 Adiponectin

Adiponectin is an adipocyte-specific secreted hormone that plays an important role in glucose and fatty acids metabolism (Díez and Iglesias, 2003). Concentrations of adiponectin seem to be higher in women than in men (Díez and Iglesias, 2003). On the other hand, they tend to be decreased in obesity and CMD in general and in visceral and central adiposity in particular. Adiponectin is therefore used as a biomarker of adipose tissue dysfunction and consequently increased CM risk (Ahl et al., 2015; Hatzis et al., 2013; Petersen and Shulman, 2018; Yaribeygi and Sahebkar., 2018).

Adiponectin concentrations also increase during periods of fasting (Fang et al., 2018) and when following a Mediterranean diet (Reis et al., 2010). Ma and colleagues (2016) found no impact of macronutrient composition of four different weight loss diets differing in fat and protein content and concluded that weight loss rather than specific macronutrients led to increased adiponectin levels. To the contrary, Yeung et al. (2010) found that even in the absence of weight loss MUFA-rich diets demonstrated the highest increases in adiponectin levels compared to CHO-rich or protein-rich diets. Fatty acids seem to have differentials effects with n-3 fatty acids and MUFA positively associated, and SFA negatively associated with circulating adiponectin levels (Haidari et al., 2014) but this occurred within the context of a HC diet. The quality of CHO also seems to be associated with circulating adiponectin levels. Low-glycaemic index CHO, fibres and wholegrains are positively and higher glycaemic index CHO and starches negatively associated with concentrations (AlEssa et al., 2016; Pischon et al., 2005).

To date a limited number of LCHF studies have reported the impact of the diet on adiponectin concentrations. Wycherley et al. (2010) found that adiponectin levels increased significantly over 12 months in 26 overweight and obese adults on a hypocaloric LCHF diet. However, this was an effect of time rather than diet and increases were similar in the

comparison group. The increase in this instance might have therefore been more attributable to weight loss rather than intake (or reduction thereof) of specific macronutrients. Adiponectin levels also increased in 18 obese adults consuming a 12-week LCHF diet in a trial conducted by Ruth et al. (2013). Standard deviation of the mean was very high so that caution has to be applied in assessing the meaning of these results. A 12-week study conducted by Hu and co-workers (2015) found that a prescribed LCHF resulted in significant increases in adiponectin concentrations at 6 months (when adherence seemed to have been at the highest level based on CHO intake) in obese adults.

#### 3.1.2.4.2 Leptin

Leptin is also an adipocyte-derived hormone, which regulates hunger and satiety and consequently influences food intake. Increased leptin levels lead to reduced appetite and food consumption and increased energy expenditure (Mechanick et al., 2018). Circulating leptin concentrations naturally fluctuate throughout a 24-hour day. From about 8:00 concentrations start to decrease, then increase again from late afternoon. Highest concentrations of circulating leptin occur during the night (Sofer et al., 2013). Leptin levels are positively associated with increasing obesity, even more so in women than in men (most likely due to greater AT stores in women compared to men) until a leptin-resistant state is reached. (Mechanick et al., 2018).

An inverse relationship between CHO intake and circulating leptin concentrations and a positive association between fat intake and SFA intake and leptin has been reported in both lean and obese men (Chu et al., 2001; Cooling et al., 1998). Cooling et al. (1998) recruited 19 lean young men, who were either classed as habitual low-fat (<31.8%E) or habitual high-fat (>45.4%E) consumers. Those classed as high-fat consumers had significantly higher leptin levels in both unadjusted and adjusted (for adiposity) models. Data from a cross-sectional sample of 268 healthy men showed significant positive associations between total fat intake and SFA intake and leptin and a significant inverse association between total CHO and leptin (Chu et al., 2001). This sample was also asked about their habitual physical activity levels. There was a significant inverse association between physical activity and leptin. Kong et al. (2009) examined the data from a cross-sectional sample of 165 overweight and obese postmenopausal, sedentary women. Dietary intake over the previous three-month period was determined via FFQ. There were significant positive correlations between SFA intake (g/d) and significant negative correlations between CHO intake (%TE) and leptin concentrations. Basic (only adjusted for energy) and further adjusted (for age and adiposity) linear regression models showed that there was a statistically significant inverse association

between CHO intake (%TE) and leptin. Positive association between fat intake (%TE) and SFA intake (g/d) and negative associations between fibre (g/d), sucrose (g/d) and fructose (g/d) and leptin were only significant in the basic model. Those women that had insulin concentrations above the median (≥17.5 μU/mL) did show significant inverse association between fibre and fructose and leptin and significant positive associations between total fat intake and SFA intake and leptin even in fully adjusted models. This showed the delicate interplay between the two hormones associated with food intake. There was a positive association between insulin and leptin concentrations with insulin driving leptin concentrations.

To date a limited number of LCHF studies has reported the impact of the diet on leptin concentrations. In the study undertaken by Volek and colleagues (2009), the overweight adults with atherogenic dyslipidaemia presented with elevated leptin levels at baseline and were probably leptin-resistant. By endpoint, leptin concentrations had significantly decreased and the significance remained after adjustments for BMI and fat mass. Brehm et al. (2009) instructed 22 obese women to follow an ad libitum LCHF diet for six months. Bradley et al. (2009) conducted an eight-week study in 12 overweight or obese adults prescribed 20%E from CHO. Both Brehm et al. (2009) and Bradley et al. (2009) found significant decreases in leptin levels in the LC group after six months and eight weeks respectively. In the Bradley study, this decrease occurred even at the higher end of CHO intake und LC conditions. A 12-week study conducted by Hu and co-workers (2015) found that a 12-months ad libitum LCHF diet resulted in significant decreases in leptin levels at six months (when adherence seemed to have been at the highest level based on CHO intake) in 54 obese adults.

#### 3.1.2.4.3 The leptin/adiponectin ratio

The leptin/adiponectin ratio (LAR) has emerged in recent years as a surrogate marker for dysfunctional AT and consequently for CMD risk. Studies have shown that the associations between (cardio)metabolic abnormalities and the LAR were stronger than for leptin or adiponectin alone (Jafari-Vayghan et al., 2015; Larsen et al., 2018; López-Jaramillo et al., 2014). Gupta et al. (2018) found the LAR to be associated with central adiposity and MetS in 523 postmenopausal South Asian women.

To the candidate's knowledge, only one study has investigated the impact of dietary intake on LAR. Jafari-Vayghan and colleagues (2015) analysed the dietary patterns from a cross-sectional sample of 150 healthy Iranian adults but found no significant correlations between any of the patterns (Western, healthy, mixed, traditional) and LAR. To examine whether the

LAR might have an impact on postprandial hyperlipidaemia Larsen et al. (2018) undertook an 8-hour oral fat tolerance test in 50 obese individuals (with and without at least one elevated CM risk marker) and 14 normal-weight controls. Subsequent receiver operating curve (ROC) analyses yielded a cut-off value for the LAR at >1.88 to detect delayed clearance of TG. Larsen et al. (2018) suggested that therefore the LAR might be a potential clinical surrogate marker to detect metabolic disturbances.

#### 3.1.2.4.5 Fibroblast growth factor 21

Fibroblast growth factor 21 (FGF21) is a hormone secreted from hepatic, adipocyte and myocyte tissue. It has gained attention in recent years because of its emerging roles in macronutrient metabolism, metabolic stress-response and CM risk prediction (Itoh, 2014; Gómez-Sámano et al., 2017; Ong et al., 2019). Independent of body composition, levels increase with age (Hanks et al., 2015). A recent systematic review and meta-analysis (Lakhani et al., 2018) found that high FGF21 concentrations were a significant risk for MetS and other cardiometabolic diseases with the hazard ratio as high as 1.7. This hormone can exert cardio-protective effects but -similar to leptin- obesity and IR seems to be associated with higher FGF21 concentrations (Staiger et al., 2017), although reference ranges have not been established yet. Concentrations are increased after acute (Kim et al., 2013; Tanimura et al., 2016) and short-term (two weeks) (Cuevas-Ramos et al., 2012) but not long-term (three-months) exercise (Yang et al., 2011). Macronutrient intake also appears to have an impact on FGF21 concentrations. If changes in circulating FGF21 concentrations are caused through dietary intake the hormone appears to be derived from the liver (Fisher and Maratos-Flier, 2016; Itoh, 2014; Pérez-Martí et al., 2017).

The majority of nutritional studies to date have been undertaken in rodents. Responses to high-fat diets varied depending on the type of fatty acid dominant in the feeding formula, with levels of FGF21 increasing more with corn oil than with fish oil (Gustavsson et al., 2009). Diets rich in polyphenols, contained in a variety of fruits, vegetables, legumes, cocoa, wine and tea, also had a positive effect on FGF21 levels in animal models (Pérez-Martí et al., 2017). Solon-Biet and co-workers (2016) used the nutritional modelling platform 'Geometric Framework' to compare 25 different diets of varying macronutrient composition and energy density in a murine model. They found that fat intake had no effect on circulating FGF21 concentrations, whereas low protein intake in combination with high carbohydrate (primarily starch and secondary sucrose) intake (with a ratio of 1:12) had the maximum effect on FGF21 levels, which were consistently higher under these conditions. Carbohydrate and fat intake combined (i.e. non-protein) had no significant effects on

concentrations leading the authors to conclude that carbohydrate intake was the main driver of FGF21 levels.

A KD consistently increases circulatory FGF21 concentrations in rodents, which are then rapidly reduced again on refeeding (Badman et al., 2007). The effects of KDs on FGF21 levels seems to be less uniform in humans and concentrations might or might not be elevated under these conditions (Christodoulides et al., 2009; Dushay et al., 2010). A number of factors relating to dietary intake seem to increase FGF21 concentrations. These include general protein restriction or deficiency in the essential amino acids leucine and methionine, long-term HC diets, acute (dose-dependent) fructose ingestion and acute CHO-overfeeding (Dushay et al., 2015; Fisher and Maratos-Flier, 2016; Lundsgaard et al., 2016; Migdal et al., 2016; Pérez-Martí et al., 2017; Solon-Biet et al., 2016). Response to fructose ingestion was even more pronounced in subjects with MetS, but acute glucose ingestion increased FGF21 concentrations only after 4 hours (Dushay et al., 2015). Gosby et al. (2016) found that an in-house four-day ad libitum low-protein, high-carbohydrate diet (Pro 10%, CHO 60%) significantly increased FGF21 six-fold compared to a high protein, moderate-carbohydrate diet (Pro 25% CHO 45%). An oral fat load challenge decreased FGF21 significantly after four hours in fasted individuals with levels returning to fasted levels after eight hours (Matikainen et al., 2012). The findings from human and rodent studies that protein restriction either on its own or coupled with excessive CHO intake led Hill et al. (2018) to conclude that FGF21 is a signalling hormone for both protein-restriction and macronutrient imbalances.

Whilst the effects of a KD on FGF21 concentrations were explored very early on in murine models, only a limited number of studies have focused on the effect of LCHF diets in humans. Christodoulis et al. (2009) reported on the effects of a 3-months KD on FGF21 concentrations in seven obese diabetic or non-diabetic individuals and found that levels decreased significantly by 42%. Participants were advised to keep SFA intake low and focus on MUFAs instead by consuming foods such as low-fat dairy, lean meats, poultry, fish and game. Unlike a conventional LCHF diet that limits milk consumption (Westman et al., 2010), daily intake of milk was prescribed to be ≥200ml and participants were also asked to consume 4-5 portions of fruit and vegetables per day with emphasis on salads and green leafy vegetables (the diet composition was described in Dyson et al., 2007). Perhaps not surprisingly, after three months only one of the participants presented with plasma hydroxybutyrate concentrations that indicated nutritional ketosis. Dushay et al. (2010) reported on FGF21 at baseline and after 12 days on an ad libitum KD in 11 normal-weight

and overweight healthy participants. Nearly all of the subjects were female. After 12 days, there were no significant changes in FGF21 concentrations. Intake of CHO was prescribed as <20 g/d, and plasma butyrate levels indicated good adherence to the diet. No further details on the prescribed diet and permitted foods were supplied. Gosby et al. (2016) reported on the change in FGF21 concentrations in 22 normal-weight subjects following three consecutive 4-d day ad libitum diets varying in protein and CHO but not fat content. Dietary fat was kept at 30% and dietary protein was 10%, 15% and 25% with the remaining energy provided by CHO (60%, 55% and 45%). Foods supplied to the participants included sweet muffins, muesli, different types of pasta, beef and vegetable pasta, Mexican wrap, Tandoori wrap, sushi, goulash, chow mein mince, curry, noodle stir fry and sweet and savoury snacks. Concentrations of FGF21 were significantly higher at endpoint when consuming the low-protein, high-CHO diet compared to day 1 and significantly higher than the endpoint levels after consuming 25% of protein for four days. Concentrations of FGF21 after four days on the 15% protein, 55% CHO diet were also significantly higher than after four days on the 25% protein, 45% CHO diet. Lundsgaard et al. (2016) conducted a cross-over trial with 9 normal-weight men who consumed in randomised orders for three days an experimental HCLF diet (80% CHO, 11% protein, 9% fat), control diet (62% CHO, 14% protein, 24% fat) and an experimental LCHF diet (10% CHO, 14% protein, 78% fat (68% USFA)). The experimental diets were also designed to provide an excess of 75% of daily energy. Compared to the control diet FGF21 concentrations were significantly increased by 803% with the HCLF diet. With the LCHF diet FGF21 concentrations were only non-significantly increased compared to the control. It has to be taken into consideration that both experimental diets provided low amounts of protein, less than the 15% that Gosby et al. (2016) used in their trial (see above). The increased levels of FGF21 under the LCHF conditions might have therefore been the result of a restriction of specific amino acids. The significant and high increases of FGF21 concentrations under the HCLF condition led to the conclusion that the excess CHO intake could be independently associated with this.

### 3.1.3 Body composition

Overweight and obesity are thought to be major risk factors for CMD (Mathew et al., 2016) and are defined as presenting with a body mass index (BMI) of ≥25 kg/m$^2$ and ≥30 kg/m$^2$ respectively (WHO, 2000). A subsequently extended definition took into account evidence of "abnormal or excessive body fat accumulation [that] may impair health" (WHO, 2018). This shift reflects mounting evidence that body composition, namely percentage, type, location and distribution of adipose tissue (AT), is an important factor in CM risk assessment rather than weight status and BMI alone (Pischon et al., 2008; Thomas et al., 2012).

Consequently, it has to be considered that also normal-weight individuals can potentially be at such increased risk (Bradshaw et al., 2013; Pajunen et al., 2011; Perez-Martinez et al., 2014; Shaharyar et al., 2015).

Two types of AT have been shown to be particularly detrimental to CM health when occurring in increased amounts. These are subcutaneous abdominal adipose tissue (SAAT), deposited around the waist (impacting waist circumference), and visceral adipose tissue (VAT), deposited around internal organs in the abdominal cavity (Coutinho et al., 2013; De Larochellière et al., 2014; Matieu et al., 2014; Romero-Corral et al., 2010; Shah et al., 2014). Visceral adipose tissue independent of total body fat seems to increase the risk of CMD and, consequently, mortality (Booth et al., 2014; Piché et al., 2018; Tchernof and Després, 2013). It has generally been accepted that an elevated waist circumference (WC) of >80 cm for women and >94 cm for men is associated with increased CM risk (WHO, 2011). The greater the increase in WC, the greater the risk of developing CM morbidity and mortality seems to be (Piché et al., 2018; Zhang et al., 2008). The volume of both SAAT and VAT increases with age (Kuk et al., 2009; Walker et al., 2014). Volume and distribution of AT is also gender-specific. Whilst women generally have larger AT stores, men have a greater tendency to store fat abdominally and viscerally. In women however, the hormonal changes of menopause also result in increased SAAT and VAT depots and consequently similar or even greater CM risk than men of similar age (Karastergiou and Fried, 2017; Palmer and Clegg, 2015; Walker et al., 2014).

More recently, other anthropometric markers of adiposity, such as neck circumference (NC), have gained attention as potential predictors of CM risk and T2DM and are found to be associated with individual contributors to MetS, such as WC, TG, GLUC and BP, and other markers of CMD (Ataie-Jafari et al., 2018; Cornier et al., 2011; Preis et al., 2010; Stabe et al, 2013).

Both SAAT and VAT, independent of BMI, can be affected by the amount and quality of CHO consumed in particular (Kaartinen et al., 2016; Malik and Hu, 2016; Sasakabe et al., 2015; Stanhope et al., 2009), different types of fatty acids and foods high in fats (Asharari et al., 2017; Fischer et al., 2015; Schlesinger et al., 2019; Schwingshackl et al., 2016) and physical activity (Ekelund et al., 2011; Kay and Singh, 2006; Kim et al., 2019; Lofley and Root, 2017).

Randomised controlled trials found that those participants prescribed a LCHF experienced (significantly or non-significantly) greater reductions in fat mass than the controls (mainly LF diets). These findings were made regardless of study population and their weight status,

and study duration and whether the diets were hypocaloric or ad libitum (Bazzano et al., 2014; Brehm et al., 2009; Foster et al., 2010; Yancy et al., 2004). A systematic review and meta-analysis of 14 RCTs (including 1416 overweight or obese adults) investigated the impact of a LC diet (<40%E) vs. control on fat mass (Hashimoto et al., 2016). Eight of the RCTs included prescribed a ketogenic diet. Hashimoto et al. (2016) found that after 12 months only the ketogenic diets were associated with a significant reduction in fat mass. Some studies have also investigated the effect of LCHF diets on WC and VAT. The CENTRAL trial was an 18-months workplace study conducted in 278 adults, who presented with increased WC or dyslipidaemia (Gepner et al., 2018). Participants were randomised to isocaloric LC or LF diets with or without exercise. After 18 months, the LC group had significantly greater reductions in WC with the effects even greater in the PA subgroup of the LC arm. VAT decreased similarly in both groups but was significantly greater when the exercise element was added to either diet. Veum et al. (2017) found no significant differences between their KD and LF groups after 12 weeks. Both groups lost equal amounts of FM, WC and VAT. After initially significantly greater decreases in the LC group (75 obese adults) in the study conducted by Bazzano and colleagues (2014) by 12 months both the LC and control group had significantly reduced their WC.

### 3.1.4 Physical activity

Physical activity (PA) has been defined as "any bodily movement produced by skeletal muscles that results in energy expenditure" (Caspersen, 1985, p. 126). Occupational activity, active travel, heavy work around the household (including gardening), active recreation and competitive sport are all classed as forms of physical activity. Exercise is a sub-category of physical activity in that it is planned, repetitive and structured and should therefore be distinct from physical activity in general (Caspersen, 1985). Physical activity levels (PAL) are an important consideration in a nutrition-context as the amount of energy expended through PA is taken into account when making recommendations on the amount of energy required by an individual (SACN, 2011).

UK Physical Activity Guidelines recommend for healthy adults to undertake at least 150 minutes of moderate-to-vigorous physical activity (MVPA) per week (Department of Health, Physical Activity, Health Improvement and Protection, 2011). Insufficient PA has long been identified as an independent risk factor for CMDs and even small amounts of PA can have preventative effects (Bull et al., 2017; Warburton and Bredin, 2017). A systematic review of 15 longitudinal studies assessing the impact of long-term PA on the risk of NCDs, which 288,724 adults (Reiner et al., 2013) found that long-term PA lowered the risk of

coronary heart disease, stroke and hypertension. Some studies specified that an additional weekly energy expenditure of 2000-3000 kcal in form of MVPA was required to demonstrate preventative effects. The same review also found beneficial effects for the reduction of risk of developing T2DM. A pooled analysis of nine population-based British cohort studies (O'Donovan et al., 2017) concluded that physical activity should be recommended to those with low HDL-cholesterol levels to reduce risk of mortality as HDL functionality might improve even in the absence of increased HDL-C concentrations. Diaz and Shimbo (2013) reviewed the evidence for the use of PA in the prevention of hypertension and confirmed that PA can be a useful tool, although concluded that further studies were needed to establish whether aerobic or strengthening PA is more beneficial, how long the PA would need to last to exert beneficial effects and the level of intensity required.

In addition to having beneficial impacts on biomarkers surrogates of CM risk, PA is also associated with adiposity. Paley and Johnson (2018) report in their narrative review that PA also reduces total and abdominal obesity even in the absence of weight loss, which means that lean mass will have increased. Physical activity in form of moderate-to-vigorous-intensity structured exercise has also been found to decrease VAT in overweight adults according to the systematic review and meta-analysis of 15 controlled and uncontrolled trials by Vissers et al. (2013). Aerobic exercise had stronger and more significant effects on VAT reduction than strength exercise. Combined exercise programmes had no significant effects on VAT reduction. The researchers also recommended that future studies take into account possible confounders such as obesity phenotype, training type and intensity and gender. Ismael et al. (2012) also came to the conclusion that aerobic exercise compared to resistance training was more effective in reducing VAT in a systematic review and meta-analysis that also included studies with normal-weight subjects. Physical Activity in general has therefore been shown to have a positive impact on a number of CM risk markers, but the type, duration and intensity are still a matter of discussion.

Physical activity of any kind requires fuel, which can either be provided in the form of glucose (CHO) or fat. Under certain conditions ketone bodies can also provide energy (Evans et al., 2017). Under normal dietary conditions CHO is the more readily available fuel, whereas fat stores are generally accessed with prolonged exercise (Cipryan et al., 2018) or when the body has been under conditions of prolonged fasting or starvation (e.g. low-calorie diets) (Evans et al., 2017). Adaptation of skeletal muscle to utilise fat as fuel can occur as quickly as five days on a LC (non-ketogenic) diet (Burke, 2015), and within 3-4 weeks on a strict ketogenic diet the human body is thought to be keto-adapted and derive

most of its fuel from fat (Volek et al., 2015). Therefore, it would have been expected for participants of the present study on the LCHF diet to have adapted to utilising fat as fuel for their activities by study interim point. Some have expressed concerns that LCHF diets result in lack of energy due to the drastically reduced CHO intake (Burke, 2015). However, according to Phinney (2004) these types of diet do not necessitate cutting back on leisure-time or occupational physical activity.

### 3.1.5 Hypothesis

The candidate hypothesised that individuals presenting with detrimental values for the CM risk markers DBP, GLUC, HDL-C, SBP, TG and/or WC, would show beneficial changes for these and other risk markers after following an ad libitum LCHF diet compared to individuals following an ad libitum higher-carbohydrate diet, such as the one recommended to the UK general population. The study was entitled "CALIBER" (Carbohydrates, lipids and biomarkers of traditional and emerging cardiometabolic risk factors).

### 3.1.6 Aims and objectives

The aim of the study was to investigate whether a low-carbohydrate, high-fat (LCHF) diet vs. a higher-carbohydrate, moderate-fat diet, as recommended in the UK dietary guidelines and represented by the EWG, would ameliorate CM risk factors in normal-weight and overweight white-Caucasian adults aged 19-64 years at increased CM risk.

***Objectives***

- Measure the impact of a well-designed LCHF diet vs. a well-designed diet based on the EWG on
    - Traditional and emerging surrogate markers of CM risk
    - Body composition and distribution of adipose tissue
    - Amount of mean moderate-to-vigorous physical activity (MVPA) undertaken
    - Food cravings
- Measure level of adherence to a well-designed LCHF vs. a well-designed diet based on the EWG in a free-living population

Primary and secondary study outcomes are presented in Table 3.1 and 3.2.

Table 3.1 – Primary outcomes of the CALIBER study

| **Primary outcomes** | **Measured via** |
|---|---|
| Plasma lipid profile (HDL-C, LDL-C, TG, small dense LDL-C)<br>Apolipoproteins A1 (apoA1) and B (apoB)<br>Fasting plasma glucose | Randox Daytona Clinical Chemistry autoanalyzer (County Antrim, UK) |
| Adiponectin<br>Leptin<br>Insulin | Randox Evidence Investigator Biochip Immuno-analyser (County Antrim UK) |
| Insulin resistance | HOMA2-IR (Homeostatic Assessment Model of Insulin Resistance) |
| Fibroblast growth factor 21 (FGF21) | Enzyme linked immunosorbent assay (R&D Systems, Minneapolis, USA) |
| Systolic and diastolic blood pressure | OMRON M2 Compact Upper Arm Digital Automatic Blood Pressure Monitor (OMRON HEALTHCARE CO., LTD, Kyoto, Japan) |
| Adipose tissue amount and distribution | Bioelectrical impedance - seca mBCA 515 (seca®, Hamburg, Germany)<br>Anthropometry - Waist circumference (tape measure)<br>Anthropometry – Neck circumference (tape measure) |

Table 3.2 – Secondary outcomes of the CALIBER study

| **Secondary outcomes** | **Measured via** |
|---|---|
| Subjective food cravings | UK Food Cravings Inventory (UK-FCI) |
| Leptin (satiety marker) | Randox Evidence Investigator Biochip Immuno-analyser (County Antrim UK) |
| Moderate-to-vigorous physical activity (MVPA) | ActiGraph GT9X (accelerometry) |
| Adherence to prescribed diets<br>Dietary intake<br>Plasma ketones | <br>Four-day food diaries<br>Randox Daytona Clinical Chemistry autoanalyzer (County Antrim, UK) |
| Experience of following well-designed LCHF or well-designed EWG diet for eight weeks | Non-validated semi-structured quantitative questionnaire |

The original contribution of this research to the existing body of evidence was firstly, that participants were randomised to a LCHF or an EWG diet (and not a LF diet), secondly, that the impact of the diets on novel surrogate markers, such as FGF21 was examined, and thirdly, that the analysis went beyond the macronutrient prescription and examined subgroups of SFA and CHO and the main food groups contributing to the intake. This was done in acknowledgement that the matrix of food items might be more impactful due to the synergetic effects of the nutrients contained within the matrix than the main macronutrient of the food alone.

### 3.2 Study design

For the CALIBER study, two groups with ten participants each were envisaged. The study was designed as a parallel randomised pilot study based on Medical Research Council (MRC) and National Institute for Health Research (NIHR) guidelines, in which a pilot study is deemed to be "vital preparatory work" (MRC, 2008, p.10). Many interventions fall short of their aims due to issues with participant adherence, recruitment and retention, all which should be explored during a pilot study to make necessary design changes prior to the main intervention (MRC, 2008). Sample sizes in other publicly funded pilot trials have contained as few as ten participants per arm (Billingham et al., 2013). Due to the explorative nature of the trial, no power calculation to determine sample size was undertaken. One group was randomised to a higher-carbohydrate, moderate fat diet in form of a standardised UK diet as recommended in the EWG for eight weeks, with ≥50%TE to be derived from CHO. The other group was asked to consume a LCHF diet with a minimum 30 g and maximum 50 g of CHO per day over the course of eight weeks (Westman et al., 2007). This amount was deemed sufficiently low to induce ketogenesis (Feinman et al., 2015) whilst regarded as safe for the participants to consume, especially taking into consideration the relatively short duration of the study (see also supporting letter and independent review of study protocol by independent Registered Dietitian – Appendix A). Participants in both groups received guidelines, portion size examples, examples of meal plans covering a two-week period and corresponding recipes as supporting tools (Appendix A). They were also given the option to join a Facebook Group as part of a range of measures offering ongoing support throughout the intervention.

To support participants during the stages of the intervention with the aim of ensuring highest level of compliance a number of behaviour change measures following the categorisation of Michie et al.'s COM-B system (2011) were employed.

- Education – increasing knowledge of dietary intake during the stages of the intervention by providing food and nutrition guidelines on the allocated diet including information on portion sizes, lists of prohibited and desirable food items, a range of recipes and example weekly meal plans. The candidate (a Registered Nutritionist) was also available to provide advice and support via telephone and email or during the four-weekly lab appointments.
- Persuasion – stimulate action and increase adherence through regular reminder text messages or emails; via the Facebook Group if relevant; and in person.

- Incentivise - expectation of reward by offering an individualised extensive dietary consultation for each participant post-intervention based on the findings (worthy of £250 per hour), and by offering high street vouchers worth £50 on completion. Participants were also entered into a final prize draw for one of them to win high street vouchers worth £200.

In addition, where the participant was not the household's Main Food Provider (MFP, as defined in NDNS 2008/09 – 2013/14), they gave consent for the MFP to also have access to the resources and to the candidate for advice. This was to ensure that the dietary instructions were followed as family support has shown to improve adherence to lifestyle and dietary interventions (Karfopoulou et al., 2016; Kelly et al., 2016). No clinical results were shared with the MFP.

Participants were asked to come in for assessment at the laboratories at IM Marsh campus on four separate occasions: for an in-person clinical screening appointment to assess final eligibility for the study, and, if eligible for and consented to the study, at three time points (baseline, interim point and endpoint) for an hour-long visit to undertake a battery of repeated measures (Figure 3.2).

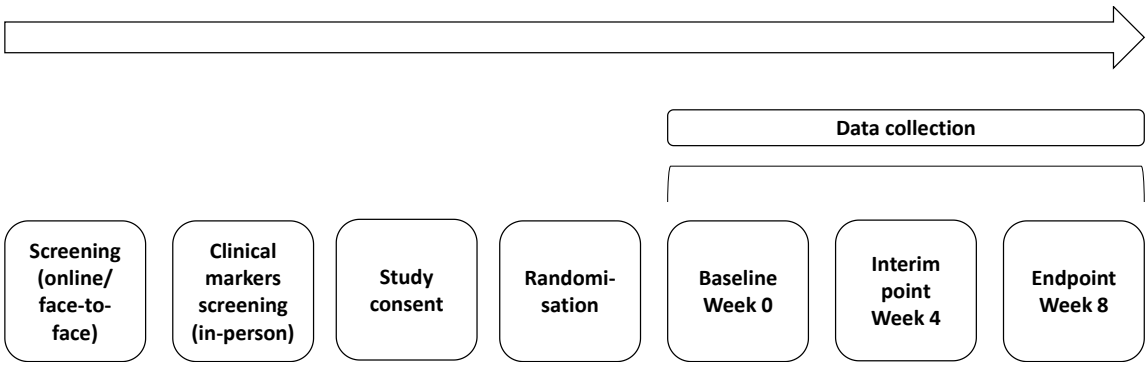


Figure 3.2. – Timeline CALIBER study

There were a number of inclusion and exclusion criteria that were applied during the screening process (Table 3.3).

Table 3.3 – CALIBER study inclusion and exclusion criteria

| Inclusion criteria | Exclusion criteria |
|---|---|
| Aged 19 – 64 years | Smoker |
| White Caucasian | Self-declared habitual vegetarian or vegan diet |
| Body mass index 18.5 – 29.9 kg/m$^2$ | Suffering from food allergies or intolerances |
| | Consuming alcohol above recommended UK government guidelines |
| | Previous diagnosis of CM disease |
| | Taking lipid, blood glucose or blood pressure-lowering medication |
| | Suffering from an eating disorder |
| | Current or previous renal impairment |

### 3.2.1 Randomisation of participants

Participants were randomised into two diet groups (A vs. B, with A being the LCHF group and B being the EWG group) in blocks of two via stratified restricted randomisation using a random number generator in MS Excel 2013™ (Sedgwick, 2012). Stratification was undertaken by sex. The sample was stratified prior to randomisation to account for the differences in male and female adipose tissue volume, distribution and hormonal differences that might affect CM risk (Karastergiou et al., 2012; Santosa and Jensen, 2014). Each stratum was restricted to six participants in each intervention group. Although total number of participants envisaged was 20, having 12 potential participants with random numbers per stratum allowed for the fact that recruitment rates and numbers for males and females might be different. Restricted randomisation was employed to ensure equal balance of intervention groups as much as possible (Higham et al., 2015; Sedgwick, 2012). An external party undertook randomisation so that the candidate was blinded to the process. Once the randomisation in MS Office Excel 2013™ was completed, the external party wrote the allocation for each participant on separate pieces of paper and sealed these in separate, numbered envelopes. There were two lots of envelopes for male and female participants. An envelope was opened each time a participant consented the study to inform them of which diet they had been allocated to. This was also the time the candidate learned of the allocation.

### 3.2.2 Design of intervention diets and participant guidance notes

All study participants were advised to follow their allocated diet ad libitum and eat until they were sated. To provide support throughout the intervention each group received guidance notes (including portion size guide) for their allocated diet, which had been written by the candidate. Participants in the LCHF group were advised to strictly limited their intake of CHO-rich foods but to consume plenty of, under the rules of the diets permitted, vegetables, preferably green (but also other colours depending on carbohydrate content), and foods containing high-quality fats, such as avocado, nuts, seeds, and permitted dairy products. Participants in the EWG group were advised to follow the UK EWG recommendations and base their diet around starchy food. They were asked to try to select wholegrain varieties when possible to increase the quality of their diet. An example of a typical week's LCHF and EWG meal plan can be found in appendix A.

Each individual participant received meal plan suggestions covering a two-week period to help them understand which foods and meals would be acceptable within their allocated diet. Participants' dietary likes and dislikes where deduced from a food frequency questionnaire (FFQ) completed and returned eight days prior to the baseline appointments. These were

taken into account where permittable within the constraints of the allocated diet to create the individual's meal plans. Recipes were sourced from LCHF websites (Atkins, n.d; Diet Doctor, n.d.) and low-carbohydrate cookery books (Creed et al., 2015; Le Roux Forslund, 2013; Noakes et al., 2016; Perlmutter, 2014) for the LCHF group and from non-LCHF cookbooks (Hemsley and Hemsley, 2014), the Change4Life website (Change4Life, n.d.) and the diabetes.co.uk website (diabetes.co.uk, n.d.) for the EWG group. In addition, recipes from UK supermarket websites (Aldi, n.d.; Tesco Real Food, n.d.) were also used and adjusted where necessary. Some EWG recipes were adjusted for the LCHF group by omitting non-permitted ingredients and vice versa. All recipes were entered into DietPlan 7 (Forestfield Software Ltd, Horsham, UK) for analysis to assess dietary composition. From there suitable two-week meal plans were created to meet the macronutrient composition of EWG (≥50% energy from CHO) or LCHF (30-50 g/d of CHO). Low-carbohydrate, high-fat diets that are not well-designed carry the risk of low fibre intake (Brouns, 2018; Tonstad et al., 2014), deemed to be detrimental to CM health (Reynolds et al., 2019). Overall, the UK population has been found to be falling short of consuming the recommended 30 g/d of dietary fibre (Bates et al., 2016; Hooper et al., 2015a). Both groups were therefore provided with a list of fibre-rich foods that were permitted within the remit of their allocated diets and a non-validated checklist of these fibre-rich foods (Appendix A) was administered at each visit to university laboratories to not quantify participants' fibre intake but to gain a general awareness whether they were consuming any of these foods.

Low-carbohydrate, high-fat diets can also be low in magnesium and potassium (Crowe, 2005; Gardner et al., 2010). As is customary in LCHF trials (Volek et al., 2009; Westman et al., 2002) to prevent any potential shortfalls in vitamin and mineral intake participants in the LCHF group were supplied with an over-the-counter multivitamin and mineral supplement (Centrum Women 50+, Pfizer Inc., N.Y., USA). This particular supplement was chosen to ensure that every potential LCHF participant was receiving the closest to the optimum for their age and gender, without putting anyone at risk of an overdose. Each participant was instructed to take one supplement tablet per day, preferably with food to aid absorption and bioavailability and the vitamins and minerals contained. The original labels of the supplement were removed from the box to blind the participants to its make and composition and replaced with a CALIBER label containing the participant ID, the number of supplements in the container and the date the container was handed to the participant, generally at the baseline and interim laboratory visit. Adherence to taking the supplement on a daily basis was checked at the next visit to the laboratory by counting the numbers of supplement tablets left in the container. The composition of the supplement is shown in

Appendix A. Potassium was not supplemented. Exceeding the recommended daily intake of 3500mg can be toxic and have severe side effects (Expert Group on Vitamins and Minerals, 2003). Due to the risk of an overdose, potassium is therefore often not at all contained in UK general multi-vitamin and mineral supplements and is by law limited to amounts of 200mg per tablet (Expert Group on Vitamins and Minerals, 2003). For cost reasons and to keep participant burden to a minimum no additional potassium food supplement was administered. The participants were instead given dietary advice regarding potassium-rich permitted foods and recipes, such as home-made bone broth. The participants allocated to the EWG group were not given any supplementation as UK dietary guidelines state that a balanced, healthy diet should not warrant the need for such (PHE, 2016a).

### 3.2.3 Ethical approval

Liverpool John Moores University's Research Ethics Committee (LJMU REC) approved the CALIBER study on 14 December 2016 (REF 16/ELS/029). The final amendment for cohort 1 was approved by LJMU REC on 11 July 2017, which requested samples to be stored for future research under the Human Tissue Act (Appendix A).

The CALIBER study was registered with ClinicalTrials.gov (NCT03257085).

### 3.3 Sampling, recruitment and Screening

#### 3.3.1 Introduction

Whilst the NDNS applied a stratified and clustered approach in their sample selection recruitment for the CALIBER study had to be much more constrained in this respect due to the shorter time frame, single location for the clinical assessments and higher participant burden. The recruitment of sufficient sample sizes within a pre-determined timescale is a particular challenge in all trials and many trials fail to meet recruitment targets (Carlisle et al., 2015; Treweek et al., 2018; Walters et al., 2017). A range of recruitment methods has been developed, which lately reflect the digitalised world we live in (see below). Success rate for these methods can vary, and it is important to establish the reasons behind their successes or failures. Having recruited sufficient numbers, although reason for celebration, does not necessarily mean that sufficient numbers will complete the study and one could say that this is a perfect example of not counting the chickens before they have hatched; post-recruitment attrition rates can be high, which would be a problem in any study. Participant retention is therefore paramount, especially if the trial is publicly funded, as high attrition can affect the validity of the results and introduce bias (Kearney et al., 2017; Routledge et al., 2017; Walters et al., 2017).

Recruitment methods for clinical trials commonly include face-to-face; word-of-mouth; posters and flyers, and social media, such as Facebook and Twitter (Frandsen et al., 2016; Whitaker et al., 2017) . In a systematic review of 35 studies that used social media for recruitment Whitaker et al. (2017) found that the majority of participants targeted were in the 16-24 age range but some included older populations. They deemed social media to be a useful tool to address hard-to-reach populations and recommended it to be integrated in recruitment strategies. However, the younger age of the study samples might not make these recommendations applicable for all studies and the conversion rate of 4% from expressing initial interest to becoming participants does not seem necessarily effective. The costs of paid advertisement on social media (‘pay-per-click’) might also make this prohibitive for some studies. Social media platforms, such as Facebook, seem to be indeed more successful for recruiting younger population as the study conducted by Loxton et al. (2015) demonstrated. For a national longitudinal study (Australia) nearly 70% of the recruited 17,069 women aged 18-23 years were recruited via Facebook and only about 5% via traditional methods. A pilot study undertaken by Nolte et al. (2015) in which participants (recruited via either Facebook, email, flyers or an in-house database) were asked to complete a web-based health-related questionnaire also showed that those recruited via Facebook and not via flyers tended to be younger. Those that were recruited via email tended to be past

retirement age. However, the most successful recruitment methods (60% of 374 participants over five months) was the online database with details of individuals who had declared a general interest in taking part in research. The vast majority of respondents was white. Overall, using the in-house system and Facebook was deemed a time and cost-effective way to recruit participants. These results are very interesting from the perspective that they confirm that the sample population targeted needs to be born in mind when considering recruitment tools and that any institution should consider building up a database of potentially interested parties as a long-term strategy. For Routledge et al. (2017) utilising an in-house system was also the most effective (and cost-efficient) recruitment tool. The majority of the participants to a web-based hypertension intervention learned about the study when phoning the hospital for other reasons. Whilst in the call queue the hospital telephone system would display a message briefly describing the study and giving a contact email address. Other recruitment methods included flyers, attending a health fair and word-or mouth. A number of online methods were also employed (including google ads, Facebook and a blog post). All online methods yielded about 43% of participants but the conversion rate from interested party to enrolled participant was far lower. Frandsen et al. (2017) also used a combination of advertising methods (flyers, radio adverts, newspaper adverts, word-of-mouth and paid advertising on Facebook) to recruit adults to a smoking cessation medication trial. In a subsequent analysis of recruitment rates they not only investigated how many potential participants each method yielded but calculated conversion rates throughout four study stages (screened, eligible, enrolled, completed). The aim was to look at the bigger picture of which method yielded the most participants that would be study completers as participant retention and adherence vitally contribute to study validity (see above). Whilst social media was a more cost effective way to get eligible respondents, it was a more expensive method when only completers were included in the analysis. Using social media compared to traditional methods did not yield significantly more participants going on to complete the study. This led the authors to the conclusion that social media might be useful for generating initial interest but ultimately less so for participant retention. These are very interesting findings that need to be taken into consideration. At these time, these will also be very specific to the sample population targeted and might therefore not apply for all trials. Frandsen et al. (2017) therefore recommend using pre-screening questionnaires as an interim step to determine eligibility prior to screening. To maximise study advertising budget they advised to use a combination of traditional and digital methods. Overall, these studies confirmed that it was best to employ a combination of both traditional and online recruitment

strategies regardless of the target population. However, some methods (paid ads) might be prohibitive due to cost reasons.

To identify reasons for difficulties in recruiting study participants Martin et al. (2013) analysed screening logs from 15 CV RCTs at a large university's medical centre over a six-year period. Both potential participant burden and participant characteristics played a vital role in recruitment and retention to these studies. Of 655 patients who were eligible for participation, only 58% consented to taking part. The others declined due to study-related reasons and participant-related reasons. Study-related reasons were participant burden through the tests undertaken during the study and the duration of the trials (>6 months). The studies would have assessed primary and secondary prevention measures of CVD. Participant-related reasons were older age and location of the study site compared to location of place of residence. Whilst the findings that participant burden, including travel time to the study site, and nature of the studies/tests are generalisable, it has to be considered that a number of the patients in this particular analysis were already suffering from acute illness and other health-related trials. Team et al. (2018) conducted 22 interviews with health professionals from a wound clinic background to examine potential reasons for low-recruitment rates to clinical trials. Study-related barriers that could also apply to the CALIBER study included the strictness of inclusion/exclusion criteria, the nature of the study/test undertaken; participant-related barriers included unwillingness to comply with the requirements of the study/low confidence in ability to adhere, location of study site (travel time) and number of appointments (unable to take this much time off work), fear of blood tests, lack of family support. Study-related enablers included changes to study protocol (inclusion/exclusion criteria, frequency of appointments); participant-related enablers included family support. The design of the CALIBER study meant that participants might be unwilling to travel to morning appointments to the university laboratories. The additional time required to collect food questionnaire templates and accelerometers only added to the participant burden. The candidate therefore needed to employ a flexible approach to the latter (e.g. meet participants at a location convenient for them to hand over the equipment). The issue of family support was addressed by consciously trying to consider the additional burden on the MFP (see chapter 2.1).

As highlighted above an important factor in strengthening a study's validity is the retention of participants, and there are a number of things that prevent attrition. Robinson et al. (2015) undertook a systematic review of the retention strategies employed by 88 trials. They found that a combination of strategies and increasing the number of strategies employed yielded

better retention rates. Due to the number of times the participants had to attend appointments at the university's laboratories and completed an array of documents, it could be said that study complexity and participant burden was high. Therefore recruitment and retention could potential be an issue over the course of the study (Newington and Metcalfe, 2014a; Newington and Metcalfe, 2014b). It would therefore take highly motivated participants and good rapport between the researcher and potential candidates to achieve optimum recruitment and retention rates (Fogel, 2018) . Generally successful retention strategies that applied to the CALIBER study are summarised in Table 3.4.

Table 3.4 – Successful retention strategies to clinical trials identified by Robinson et al. (2015) and employed in the CALIBER study

| **Retention strategy** | **CALIBER study** |
|---|---|
| Assign one primary clinical researcher to each participant | All participants were assessed during the clinical appointments by the candidate. |
| Provide nonfinancial incentives or tokens of appreciation | Each completer was offered a consultation based on their results from the study. |
| Provide financial incentives | Each participant was given a £50 high-street voucher on completion and was entered into a prize-draw to win an additional £200 worth of high-street vouchers. |
| Provide reminders about appointments and study participation | Each participant received regular appointment and meeting reminders and reminders to fast prior to the clinical appointments. |
| Explain study requirements and details, including potential benefits and risks, to participants<br>Specify the schedule of visits<br>Provide realistic estimates of the schedule for visits and time for each visit<br>Spend time answering participants' questions | Each participant received a participant information sheet with details on the assessments that would be carried out at each study visit and the time each visit would approximately take. The candidate was available to answer any questions and appointments were scheduled in a manner that allowed time for a chat with participants if they wished so to enquire about any problems that they might encounter with adhering to the diets. |
| Provide relevant informational resources such as books or pamphlets | Each participant was given extensive guidance notes on the diet they were allocated to and example meal plans. The candidate was also available to provide additional recipes should participants want them. |

For initial recruitment, the CALIBER study aimed at recruiting 20 participants to be randomised to the two dietary intervention group via a combination of recruitment methods. This reflected the fact that the study sample was to be recruited from a wide age range (19 – 64 years) and included both male and female participants.

### 3.3.2 Sampling and Recruitment

To recruit participants for the CALIBER study a mix of non-probability sampling methods was applied. These included purposive homogenous sampling, convenience sampling and snowball sampling (Martinez-Mesa et al., 2016). As determined by the inclusion and exclusion criteria defined for study participants (chapter 3.2) the aim was to recruit a sample that was a homogenous as possible. The selection of these convenience and snowball sampling methods was due to time- and budget constraints. Further details on how these methods were implemented can be found below. Although non-probability sampling methods limit the generalisability of the results, they are commonly used in pilot and feasibility studies, such as CALIBER (Kandola et al., 2014).

A number of recruitment methods were used to find participants for the CALIBER study, which could be categorised as active and passive methods (Raynor et al., 2009). Active methods include those in which candidates are identified by the research team, passive methods include those were the candidates are self-identified (see Table 3.5).

Table 3.5 – Types of recruitment methods used in CALIBER study

| Active recruitment | Passive recruitment |
|---|---|
| Face-to-face | Social Media (Facebook, Twitter, Next Door Mossley Hill) |
| | Emails |
| | Word-of-mouth |
| | Posters |
| | Flyers |

Recruitment was undertaken in the Greater Merseyside area via face-to-face contact, email distribution, distribution of posters or flyers or posts on social media (Facebook, Twitter and Nextdoor Mossley Hill, a social media platform for the Mossley Hill postcode area (a district in Liverpool, Merseyside)) available free of charge to inhabitants of this neighbourhood on an opt-in basis).

Face-to-face recruitment took place on several occasions on two campuses of LJMU (IM Marsh and Byrom Street), at a local superstore (Tesco, Mossley Hill) and at the Liverpool Rock'n Roll marathon event in May 2017. Candidates completed paper-based versions of a screening questionnaire (Appendix A) to determine eligibility for study participation. The superstore was chosen as a recruitment venue due to its proximity to the university campus increasing the likelihood that shoppers at this particular location would live in close proximity to campus reducing travel time and participant burden. The Liverpool Rock'n Roll marathon is an annual event, which attracts not only those competing in the different runs but a large number of spectators.

After request by the research team, a university administrator with access to a central mailing list sent an email invitation to all staff and students at LJMU. The same email invitation was sent to potential candidates who had heard about the study through word-of-mouth via friends or colleagues and subsequently requested further information.

Researchers from the BBC programme "Trust me I am a doctor" forwarded the recruitment flyer to their mailing list of previous BBC study participants who had opted into further communications. Any interested party could contact the CALIBER researcher directly via work mobile phone or email. If preliminary eligibility was demonstrated potential participants were then sent the participant information sheet (PIS) with further study information and could decide whether to pursue further contact with the study organiser.

Posters and flyers were displayed and posted across LJMU campuses, at Liverpool Hope University campus, the local community around IM Marsh campus in Mossley Hill, and several Liverpool city council libraries. These flyers contained a link to an online screening questionnaire (see below) and the candidate's contact details.

A Facebook page was created for the CALIBER study, which contained a link to the online version of the screening questionnaire hosted via Bristol Online Surveys (now Online Surveys) that candidates had to complete. The online screening questionnaire was set up in a way that at several stages respondents not meeting the inclusion or meeting an exclusion criterion were 'screened out' and presented with a message thanking them for their time.

In addition, participants coming in for clinical screening (see below) were asked if they knew of other people that might be interested in taking part in the study. If that was the case participants were encouraged to pass information on the study onto these individuals. Recruitment commenced on 23 January 2017. The online questionnaire closed on 30 June 2018. Face-to-face recruitment ended on 18 July 2017. In total recruitment was open for 177 days. A flow chart of the recruitment process with reasons for exclusion of respondents can be found in Figure 3.3.

The majority of people initially contacted or asking for further information did not state a reason for their non-response or decision not to take part. Those that did expressed their unwillingness to follow a specific diet for the allocated length of the study or that they would not be able to attend the number of appointments required. Whilst initial recruitment might depend on study complexity and duration, study retention might depend on participant-specific factors (see above). Those participants that were recruited onto the study were able to fully participate and allocate the time required for the study (including attending all

necessary appointments) because their employment status and type of employment permitted them to do so. They were either (semi-)retired, worked in close proximity to the university laboratories (for example because they lived nearby and worked from home or were members of the university), or worked flexible hours or shifts. Reasons stated for taking part in the study were health improvement and weight loss. All of the participants consenting to take part completed the study (see figure 3.3).

With a total recruitment duration of 177 days (5.9 months) recruitment rate was 1.69 participants per month. With final recruitment achieved only being 50% of the initial target, the CALIBER study stands not alone with 44% of publicly funded trials failing to meet their recruitment target. Its average recruitment rate was, however, higher at 1.69 per month compared to a median of 0.92 achieved by 151 published RCTs, which received funding from the UK NIHR HTA between 2004 and 2016. A retention rate of 100% also put the CALIBER trial above 89% for the 151 RCTs (Walters et al., 2017) .

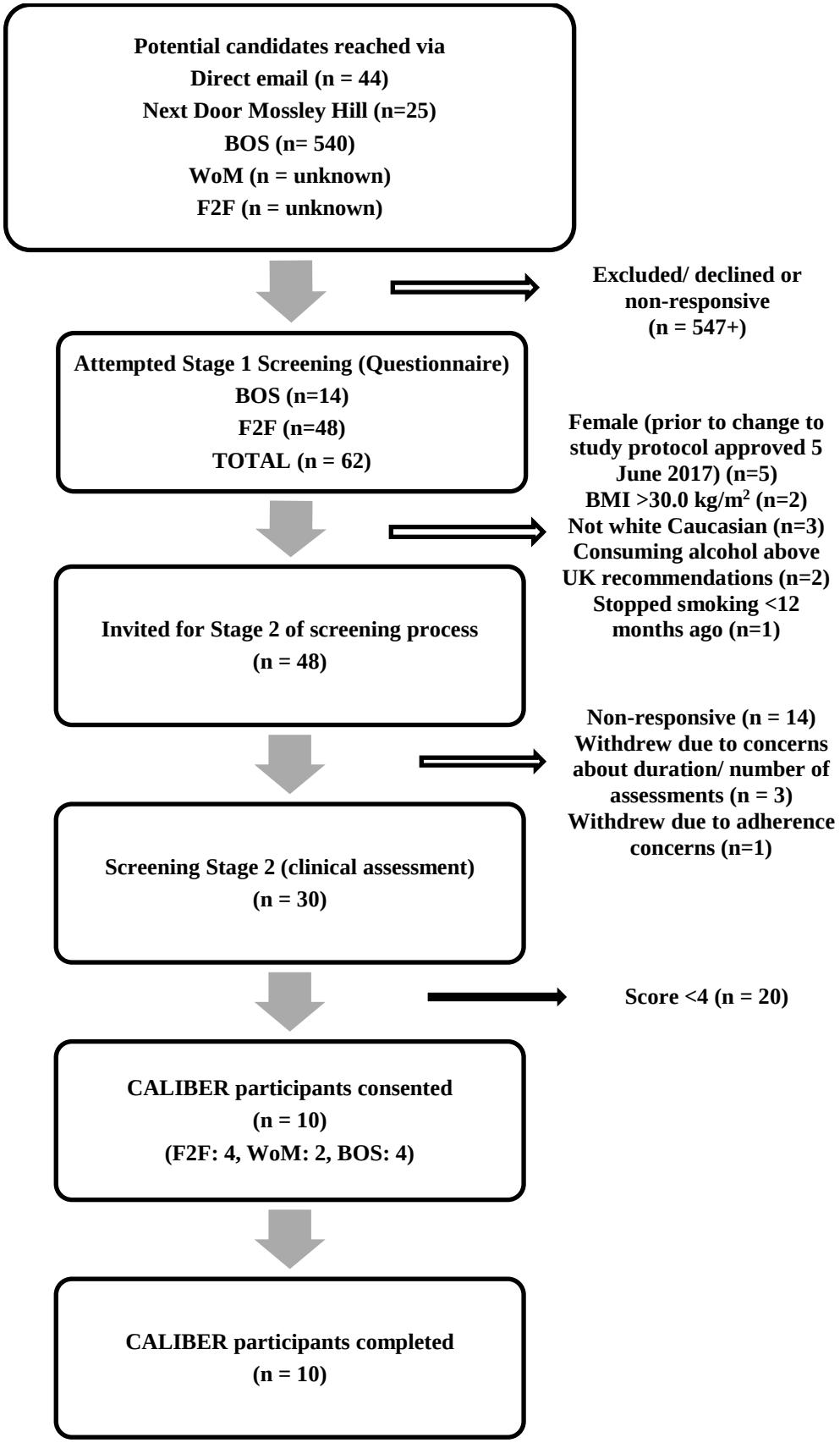


Figure 3.3 – Flowchart illustrating the CALIBER recruitment process
BOS – Bristol Online Survey; F2F – face to face; WoM – word of mouth

### 3.3.3 Screening and consent

All candidates had to provide their contact details at the end of both the online questionnaire and the paper-based version. Those that successfully completed the first stage of the screening process were invited to come to the university laboratories at IM Marsh (Mossley Hill, Liverpool) for an in-person clinical screening appointment to confirm study eligibility. A flow cart detailing the clinical screening process can be found in Figure 3.4. First respondents had their height and weight measured. The candidates' height was measured using a stadiometer according to protocol used in the NDNS RP. Briefly, participants were asked to remove their shoes and were asked to stand with their feet flat on the floor, feet together and heels against the stadiometer rod. Their back was as straight as possible, their arms hanging loosely by their sides and they were asked to face forwards. Their head was moved so that they were facing straight ahead and the head plate of the stadiometer was resting on the crown of the head. Candidates were asked to breathe in deeply and stretch to their fullest height. The head plate of the stadiometer was adjusted if necessary and participants were asked to step away from the stadiometer without ducking their head. It was checked that the head plate had not moved during this movement. The height reading was taken of the measuring scale to the nearest millimetre. Weight was assessed using the scales of a bioelectrical impedance scale (mBCA seca 515 (seca®, Hamburg, Germany)) and the body mass index (BMI) was read from the screen of the seca after inputting of the height measurements. If the BMI was ≥30.0 kg/$m^2$ it was calculated manually after the following adjustments. A clothing allowance of 1.2 kg for men and 0.8 kg for women was applied (Whigham et al., 2013). Height and weight were used to calculate the body mass index (BMI) as the body weight (kg) divided by the squared height ($m^2$). The BMI was assessed to verify that the candidate fell indeed within the normal-weight or overweight category (18.5 – 29.9 kg/$m^2$). If they were within the included BMI range, a fasted blood sample was taken next. This was done via finger prick (Alere Cholestech LDX® Analyzer (Alere San Diego Inc. USA)), a commonly used and accurate screening method in research and point-of care environments , which is also less invasive Bastianelli et al., 2017). Only a small amount of blood was taken (40 $\mu$L) and participants experienced only mild discomfort. The sample was taken according to manufacturer's instructions. Briefly, the respondent was asked to sit quietly for five minutes before sample collection. It was ensured that the fingers and hands were warm to the touch as this aided the ease of taking the sample. Otherwise, the respondent's finger was gently massaged from the base to the tip several times to ensure blood flow to the tip of the finger. The sample was collected from a spot on the side of one of the middle fingers from the hand preferred by the respondent. The site was cleaned with

an alcohol swap and cleaned with a gauze pad before the finger prick. The spot was pierced with the lancet and a large blood drop squeezed out. This first drop was wiped away and the second drop was collected after the finger was squeezed again and held downwards. The blood was collected in the equipment's capillary tube and placed into the cassette well. The excess blood was wiped from the finger and the respondent was asked to apply pressure until the bleeding stopped. A 5% error margin, which is commonly accepted in participant screening and reflects potential inaccuracies of medical and clinical devices was permitted Warnick et al., 2008).

Next blood pressure was measured three times using a sphygmomanometer (OMRON M2 Compact Upper Arm Digital Automatic Blood Pressure Monitor, OMRON HEALTHCARE CO., LTD, Kyoto, Japan) after respondents had been seated and at rest for five minutes. From the three measurements each obtained for systolic and diastolic blood pressure an average value was calculated. Waist circumference (WC) (defined as the midway point between the iliac crest and the costal margin) was then measured three times to the nearest mm according to the protocol applied in the NDNS RP using a tape measure calibrated in mm. Briefly, participants were asked to relax, but stand erect, and breathe normally with feet about 25 to 30 cm apart and arms hanging loosely at their sides. Weight was evenly distributed between both legs. The average of the three measurements was calculated.

Respondents were invited to join the study if they had a score of ≥4 from a combination of risk markers based on a point system developed by Jebb et al. (2010) , which was slightly adjusted to include only markers accessible through point-of-care testing. As these markers could all be obtained during the screening appointment participant burden was kept to a minimum at this point. This scoring system reflected that the participants could be at mild risk of pre-metabolic or metabolic syndrome (dependent on whether values for 3 out of 5 of these markers were increased (Vidigal Fde et al., 2015; Yin et al., 2013).

Respondents would have been excluded from the study and advised to see their GP if certain thresholds had been exceeded during the screening process. None of the candidates had to be excluded from the study for this reason. Details of the scoring system and exclusion thresholds are given in Table 3.6.

Table 3.6 – Scoring system to determine eligibility for CALIBER study*

| Risk marker | Value | Score** | Exclusion and advice to see GP |
|---|---|---|---|
| **HDL-C** | <1.0 mmol/L (men)<br><1.3 mmol/L (women) | 2 | n/a |
| **TG** | >1.3 mmol/L | 1 | ≥ 5.7 mmol/L (increased risk of pancreatitis) |
| **GLUC** | >5.5 mmol/L | 3 | ≥ 7 mmol/L (diabetes threshold) |
| **DBP** | >85 mmHg | 1 | ≥ 100 mmHg (considerably raised) |
| **SBP** | >130 mmHg | 1 | ≥ 160 mmHg (considerably raised) |
| **WC** | >102.0 cm (men)<br>>88 cm (women) | 2 | n/a |
| **WC** | >94.0 cm (men)<br>>80.0 cm (women) | 1 | n/a |

DBP – diastolic blood pressure; GLUC – fasting blood glucose; HDL-C – high-density lipoprotein cholesterol; SBP – systolic blood pressure; TG – triglycerides; WC – waist circumference; *based on Jebb et al. (2010); **a total score of ≥4 was the threshold for study participation

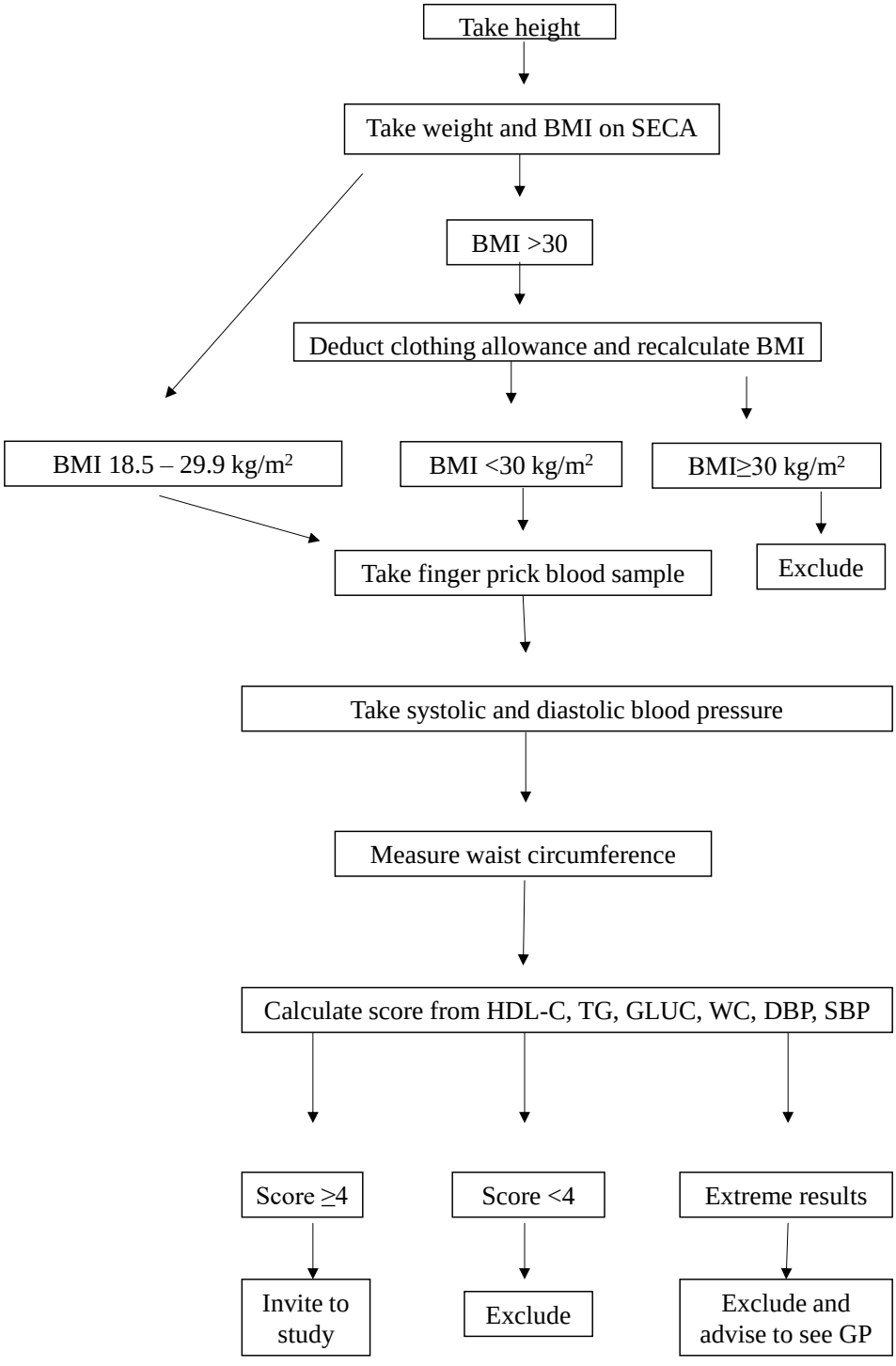


Figure 3.4 – Flowchart of screening process to determine respondents' eligibility for participation in CALIBER study

Once found to be eligible for the study respondents had 24 hours to consider whether they would like to take part in the study. On consent they were given a PIS (in duplicate – one copy was retained by the candidate (Appendix A)), informed consent form (in duplicate – one copy was retained by the candidate (Appendix A)), a very-low carbohydrate disclaimer (in duplicate – one copy was retained by the candidate (Appendix A)), a participant contact details form, a food frequency questionnaire (FFQ) (EPIC Norfolk version 6; accessible at http://www.srl.cam.ac.uk/epic/epicffq/EPIC_HC3_FFQ_V6_OCR_Uncoded.pdf (Appendix A)) and the RPAQ (see chapter 2.2.2) to complete. They were then informed of which diet they had been randomised to (see chapter 3.2.1). Further details on the analysis of the FFQ and the RPAQ can be found in chapter 3.4.1.1 and chapter 3.4.9.1 respectively.

### 3.3.4 Final sample

The final total number of participants was ten. After randomisation, there were five participants in each intervention group. Each group consisted of four men and one women. Participants allocated to the LCHF group had significantly higher BMIs and a significantly higher WC. However, this happened by chance, as all participants underwent the same process of randomisation (chapter 3.2). Information on characteristics for the two groups at point of screening are contained in Table 3.7.

Table 3.7 – Group differences at point of screening (mean and (standard deviation))*

| Group | Age (years) | BMI ($kg/m^2$) | WC (cm) | DBP (mmHG) | SBP (mmHG) | GLUC (mmol/L) | TG (mmol/L) | HDL-C (mmol/L) |
|---|---|---|---|---|---|---|---|---|
| **LCHF** | 46.0 (8.5) | 29.5 (1.0) | 104.7 (7.4) | 86.0 (7.2) | 134.0 (9.7) | 5.3 (0.6) | 2.0 (0.8) | 1.2 (0.1) |
| **EWG** | 53.0 (5.4) | 26.9 (1.8) | 91.9 (5.3) | 89.9 (9.2) | 136.0 (14.2) | 5.6 (0.6) | 2.1 (0.6) | 1.2 (0.6) |
| **p** | .157 | **.024[a]** | **.015[a]** | .680 | .801 | .464 | .868 | .872 |

BMI= body mass index; DBP = diastolic blood pressure; EWG – Eatwell Guide; GLUC = blood glucose; HDL-C = high-density lipoprotein cholesterol; LCHF – low-carbohydrate, high-fat; SBP = systolic blood pressure; TG = serum triglycerides; WC = waist circumference; *Independent samples t-test, [a]significant at $p < .05$

### 3.4 Methods used for data collection and analysis

#### 3.4.1 Assessing dietary intake

##### 3.4.1.1 Assessing habitual pre-study dietary intake

Food Frequency Questionnaires are a commonly used tool in dietary assessment to explore the potential association between dietary intake and disease-specific risk markers, such as those for CM risk (Shim et al., 2014) . They have also shown correlations with overall food preferences as demonstrated by the frequency of consumption of specific food items (Drewnowski and Hann, 1999) and have previously been used to assess food preferences in the context of a suitable diet (McVay et al., 2014). On completion of the initial screening process, participants were asked to complete a validated, semi-quantitative FFQ, the EPIC-Norfolk FFQv6 (available at http://www.srl.cam.ac.uk/epic/epicffq/EPIC_HC3_FFQ_V6_OCR_Uncoded.pdf (Appendix A)). This 130-item questionnaire assessed habitual dietary intake over the previous 12-months period. The questionnaire was divided into two parts, the first one containing 130 food items regularly consumed as part of a standard UK diet, the second part asking more in-depth questions about particular food items, such as fats, cereals and milk. Food item consumption frequencies in part 1 were coded from 'Never or less than once/month' (1) to '6+ per day' (9). Participants returned the FFQ eight days before their baseline visit to the laboratories. This information was used to inform their final meal plan - to be handed out at the baseline visit. The completed FFQ was analysed using open source, cross-platform software specifically developed for this purpose by the European Prospective Investigation into Cancer and Nutrition (EPIC) research team, FETA (Food Frequency Questionnaire European Prospective Investigation into Cancer and Nutrition Tool for Analysis). This tool calculates average daily total energy consumption and dietary intake of macro and micronutrients of main interest in a public health context (Fallaize et al., 2017) over the previous 12-month period. Details of the nutrients assessed can be found in Table 3.8 The tool is freely available under open government licence at http://www.srl.cam.ac.uk/epic/epicffq. The author would therefore like to acknowledge the contribution of the staff and participants of the EPIC-Norfolk Study. EPIC-Norfolk is supported by the Medical Research Council programme grants (G0401527, G1000143) and Cancer Research UK programme grant (C864/A8257).

Table 3.8 – Macro- and micronutrients and energy intake data available for analysis through Food Frequency Questionnaire European Prospective Investigation into Cancer and Nutrition Tool for Analysis

| Nutrient | Units |
|---|---|
| **Energy intake** | |
| Energy_kcal | kcal |
| Energy_kj | kJ |
| **Macronutrients** | |
| Alcohol | g |
| Carbohydrate - total | g |
| Carbohydrate - starch | g |
| Carbohydrate - sugars (total) | g |
| Carbohydrate - lactose | g |
| Carbohydrate - maltose | g |
| Carbohydrate - sucrose | g |
| Carbohydrate - fructose | g |
| Carbohydrate - galactose | g |
| Carbohydrate - glucose | g |
| Englyst Fibre - Non Starch Polysaccharides (NSP) | g |
| Fat - total | g |
| Monounsaturated fatty acids (MUFA - total) | g |
| Polyunsaturated fatty acids (PUFA - total) | g |
| Saturated fatty acids (SFA - total) | g |
| Cholesterol | mg |
| Protein | g |
| Nitrogen | g |
| **Micronutrients** | |
| Alpha carotene | mcg |
| Beta carotene | mcg |
| Calcium | mg |
| Carotene - total (carotene equivalents) | mcg |
| Chloride | mg |
| Copper | mg |
| Iron | mg |
| Total folate | mcg |
| Iodine | mcg |
| Magnesium | mg |
| Manganese | mg |
| Niacin | mg |
| Potassium | mg |
| Phosphorus | mg |
| Selenium | mcg |
| Sodium | mg |
| Vitamin A - retinol | mcg |
| Vitamin A - retinol equivalents | mcg |
| Vitamin B2 - riboflavin | mg |
| Vitamin B1 - thiamin | mg |
| Vitamin B12 - cobalamin | mcg |
| Vitamin B6 - pyridoxine | mg |
| Vitamin C - ascorbic acid | mg |
| Vitamin D - ergocalciferol | mcg |
| Vitamin E - alpha tocopherol equivalents | mg |
| Zinc | mg |

#### 3.4.1.2 Assessing ad-hoc dietary intake during the intervention

Participants were instructed to complete a four-day estimated food diary (FD) on three separate occasions, at baseline, interim point and endpoint. Participants collected the templates eight days prior to their visits to university laboratories and returned the completed diaries during their laboratory appointments. Participants were asked to record everything they ate and drank at home and outside the home on the same four consecutive days, including two weekend days (Yancy et al., 2010). The food diary template (Appendix A) was based on the NDNS RP 2008/09 – 2013/14. The pages containing two example days were omitted as the candidate went through the template with the participants prior to their baseline visit. During the first collection visit the candidate took participants through the diary template, explaining and showing examples of food portion sizes and household measures using the illustrations in the food diary template. Participants were asked to provide as much information as they could on portion sizes, for example weights given on food packaging of food items consumed. In addition to taking photographs of their meals, participants were encouraged to collect or photograph the packaging of foods consumed (containing information on weight, portion sizes and nutrition labels) and to bring with them to their next laboratory appointment or to provide electronically (via email or USB stick). To further aid the accuracy of the dietary assessment records participants were asked to record brand names of the foods consumed. The FD template also provided space to record whether they had eaten everything and if not, how much they had approximately left on the plate. Participants could also report on whether they felt that that particular day had been a typical day in terms of food consumption. The food diaries were briefly checked during the participants' visit to the laboratories at interim and endpoint to pick up on any potential issues regarding completion. For each eating occasion there was the opportunity to record whether and who else was present and where the food was consumed, including for example in front of the TV or at the table.

All four-day estimated FDs were analysed using Dietplan 7 dietary assessment software (Forestfield Software Ltd, Horsham, UK). The first meal (food and drink) consumed prior to 12:00 was categorised as meal 1 (breakfast), items consumed between 12:00 and 14:00 were categorised as meal 2 (lunch) and items consumed between 18:00 and 20:00 were categorised as meal 3 (dinner). All other food and drink items were categorised as snacks and collectively entered as meal 4. Where participants had given portion sizes in grams/ounces or household measures (e.g. 'large mug'), these were entered into Dietplan. When brand names of purchased foods or food labels were supplied, the ingredients list and portion size guides provided by the manufacturer or retailer in the analysis were used. Where

the participants provided photographic evidence of meals consumed, the food portion guides, which had been incorporated into Dietplan, were used. Where the candidates provided their own recipes, they were asked to the state the number of portions the recipe would make.

### 3.4.2 Assessing adherence to diets

Dietary adherence was assessed by two methods. Firstly, the analysis from the four-day FDs (see above) completed at interim and endpoint was used to check whether the LCHF group had a mean daily CHO intake between 30 g and 50 g and whether the EWG group derived an average of 50% of their daily total energy from CHO (Brehm et al., 2009; Desroches et al., 2013; McVay et al., 2015). Secondly, plasma ketone concentrations (β-hydroxybutyrate) were used to verify whether the participants in the LCHF group reduced their CHO intake sufficiently to enter nutritional ketosis (Hu et al. 2016; Johnston et al., 2006). The blood plasma of the EWG group was also analysed to ensure that none of the participants in this group had been tempted to follow a LCHF diet instead whilst taking part in the intervention. Hydroxybutyrate is the most abundant ketone present in blood (Newman and Verdin, 2014) and has been found to be fairly stable (Fritzsche et al., 2001; Lloyd et al., 1978). A cut-off threshold of ≥0.5 mmol/L and an upper limit of 5.0 mmol/L was used to determine whether participants had entered nutritional ketosis (Harvey et al., 2018; Volek and Phinney, 2011). Blood plasma was collected and processed as described in chapter 3.4.6.1 and ketone concentrations were analysed on a Randox Daytona clinical chemistry auto-analyser using a RANBUT D-3-Hydroxybutyrate kit (RANDOX Laboratories Ltd., Antrim, United Kingdom). To check precision of the reagent 15 repeat samples of the calibrator were analysed in a within run test. Percentage coefficient of variation (%CV) was 1.36% and well within the target %CV of <2.00.

### 3.4.3 Assessing food cravings

Participants completed a validated 24-item food cravings questionnaire (British Food Cravings Inventory (FCI-UK) (Nicholls and Hulbert-Williams, 2013) (Appendix A)) at baseline, interim point and endpoint prior to attending the laboratory appointment. The questionnaire contained three different questions. Using a Likert scale from 0 (Never) to 4 (Always/Almost every day), participants rated in the first question how often they craved foods from four different categories over the previous month. Details of the food categories can be found in Table 3.9. The second question investigated how often they consumed the above food items, with possible answers being Never = 0, Rarely (once or twice) = 1, Sometimes = 2, Often = 3, Always/Almost every day = 4. The third question asked how difficult it was to resist cravings for particular foods. Answers to choose from were Very

easy = 0, Easy = 1, Neutral = 2, Difficult = 3, Very difficult = 4. In line with the literature, answers were coded from 1 to 5 for analysis.

Table 3.9 – Food categories examined in UK Food Cravings Inventory (FCI)*

| Food category | Food items included |
|---|---|
| FCI-S (sugary foods) | Cake, chocolate, biscuit, ice-cream, popcorn, sweets, ice lolly |
| FCI-F (high-fat foods) | Sausage, hot dog, bacon, steak, gravy |
| FCI-FF (fast foods) | Fast food, fries, burger, pizza, fried chicken |
| FCI-CHO (starchy foods) | Pasta, baked potato, curry, rice, mashed potato, pasty, bread |

*Nicholls and Hulbert-Williams, 2013

### 3.4.4 Assessing occurrence of adverse events in the LCHF group

Participants in the LCHF group completed a semi-qualitative questionnaire (Appendix A) at interim and endpoint reporting on the occurrence of adverse events commonly associated with LC diets that they might have experienced since the previous visit to university laboratories. They were asked to confirm whether or not they experienced 'Impaired cognition/brain fog', 'dizziness', 'constipation', 'headaches or other flu-like symptoms', 'muscle cramps', 'bad breath' or 'general weakness'. For any adverse effect that might have been experienced participants were asked to provide details on the occurrence, such as time of occurrence, duration, severity and what they had done to ameliorate the effects. Every time an occurrence was reported, the doctoral candidate wrote and submitted an adverse events report to LJMU REC detailing what action was undertaken or which advice was given to the participant.

### 3.4.5 Assessing the participants' experience with the intervention

At their endpoint visit, participants were interviewed using a non-validated semi-quantitative questionnaire to record experiences with the intervention and rate on a Likert scale whether specific aspects were for example "a lot easier", "easier", "about the same", "more difficult" or "a lot more difficult" than their pre-study diet (Appendix A). This served the purpose of establishing potential reasons why participants might or might not have adhered to their diets and inform the design of future research studies. Areas covered by the questionnaire included practical experience with the diets (how easy to follow; how easy to shop for; how easy to cook for; how easy to follow in home environment; how easy to follow when socialising; how expensive) and psychological and health-related experiences (levels of happiness, healthiness, alertness, sleep and energy levels).

### 3.4.6 Clinical markers of cardiometabolic risk

#### 3.4.6.1 Blood plasma markers

Venous blood samples were taken from the 10 participants at baseline, interim point and endpoint after a 12-hr overnight fast and collected in BD Vacutainer® EDTA (Ethylenediaminetetraacetic acid) tubes (Becton, Dickinson and company, NJ, USA). Participants were advised that they should drink some water prior to obtaining their blood sample but to abstain from alcohol and any strenuous physical activity for 24 hours before their visit to the university laboratories. Samples were kept on ice, separated by centrifugation at 3000 RPM for 15 min at 4°C (MSE Mistral 200R, MSE, Healthfield UK) within approximately 90 minutes after collection, aliquoted into 1.7ml microtubes, frozen immediately and stored at -80°C. Maximum time between sampling and analysis was six months, during which time the proteins in the samples would have most likely kept their integrity (Mitchell et al., 2005). For analysis all samples were thawed at room temperature (Lee, 2015; Paltiel et al., 2008). The process of slow-freezing and fast-thawing that was applied in the present study was found to keep plasma samples integrity (Cao et al., 2003).

Lipids, lipoproteins and glucose were assessed via Randox Daytona clinical chemistry auto-analyser (RANDOX Laboratories Ltd., Antrim, United Kingdom). To check precision of the reagent 15 repeat samples of the calibrator were analysed in a within run test. The percent coefficient of variation (%CV) was expected to be <2.0. Insulin, leptin and adiponectin were analysed via sandwich chemiluminescent immunoassay technology (Randox Evidence Investigator™, Randox Laboratories, Co. Antrim). Fibroblast growth factor 21 was analysed using a Quantikine® enzyme-linked immunosorbent assay (ELISA) Kit (DF2100, R&D Systems, Inc., Minneapolis, USA) for FGF21 levels according to manufacturer's instruction (Gosby et al., 2016). Samples were measured at $OD_{450}$ using a CLARIOstar® high-performance microplate reader (BMG LABTECH GmbH, Ortenberg, Germany). All samples were analysed in duplicate for insulin, leptin adiponectin and FGF21, and samples from the same participant were analysed in the same batch (Song et al., 2016). All the manufacturers' recommended calibrators and quality controls for each analyte were used. Details of the analysis methods, assay sensitivity, the intra- and inter-assay %CVs, ranges and results from the between run can be found in Table 3.10.

Table 3.10 – Methods and equipment used to analyse biomarkers in CALIBER study

| Analyte | Method (kit used) | Equipment | Assay sensitivity (mmol/L) | Intra-assay %CV | Inter-assay %CV | Range (mmol/L) | Within run %CV |
|---|---|---|---|---|---|---|---|
| **HDL-C** | Direct Clearance (Randox CH3811) | Randox Daytona clinical chemistry auto-analyser (Randox Laboratories Ltd., Antrim, UK) | 0.189 mmol/L | 1.45-3.11 | 2.40-2.81 | 0.53 – 2.31 mmol/L | 1.25 |
| **LDL-C** | Direct Clearance (Randox CH3841) | | 0.189 mmol/L | 1.47-2.99 | 1.58-2.50 | 0.83 – 5.41 mmol/L | 1.09 |
| **TG** | GPO-PAP method (Randox TR3823) | | 0.134 mmol/L | 1.55-3.29 | 1.33-3.51 | 0.42 – 4.16 mmol/L | 1.14 |
| **sdLDL-C** | s LDL-EX "SEIKEN" (Randox S62616) | | Not defined for analysis via Daytona | | | | 1.54 |
| **apoA1** | Immunoturbidi-metric immunoassay (Randox LP3838) | | 6.5 mg/dl | 2.67-4.10 | 3.18-3.22 | 120 – 176 mg/dL | 0.78 |
| **apoB** | Immunoturbidi-metric immunoassay (Randox LP3839) | | 11.2 mg/dl | 1.65-4.13 | 1.79-2.73 | 47 – 157 mg/dL | n.o. |
| **GLUC** | GLUC-PAP (Randox GL3815) | | 0.335 mmol/L | 1.96-4.48 | 3.51-5.87 | 3.9 – 11.6 mmol/L | n.o |
| **INS** | Sandwich chemiluminescent immunoassay (Metabolic Syndrome Array I) | Randox Evidence Investigator ™ (Randox Laboratories, Co. Antrim, UK) | 2.32 µlU/ml | 7.5-9.4 | 9.0-14.0 | 0-300µlU/ml | n/a |
| **LEP** | | | 1.10 ng/ml | 4.6-7.7 | 6.0-8.7 | 0-100ng/ml | |
| **ADPN** | Sandwich chemiluminescent immunoassay (Metabolic Syndrome Array II) | | 164 ng/ml | 8.2-9.6 | 9.7-12.4 | 0-40,000 ng/ml | |

ADPN – adiponectin; apoA1 – apolipoprotein A-1; apoB – apolipoprotein B; GLUC – glucose; HDL-C – high-density lipoprotein cholesterol; INS – insulin; LDL-C – low-density lipoprotein cholesterol; LEP – leptin; n.o. – not obtained; sdLDL-C – small dense low-density lipoprotein cholesterol; TG – triglycerides; %CV – percent of coefficient of variation

Table 3.10 – Methods and equipment used to analyse biomarkers in CALIBER study (cont.)

| Analyte | Method (kit used) | Equipment | Assay sensitivity (mmol/L) | Intra-assay %CV | Inter-assay %CV | Range (mmol/L) | Within run %CV |
|---|---|---|---|---|---|---|---|
| **FGF21** | Quantikine® Enzyme-linked immunosorbent assay (ELISA) (DF2100, R&D Systems, Inc., Minneapolis, USA) | Measured at $OD_{450}$ using CLARIOstar® high-performance microplate reader (BMG LABTECH GmbH, Ortenberg, Germany) | 8.69 pg/mL | 2.9-3.9 | 5.2-10.9 | 31.3 – 2000 pg/mL | |

FGF21 – Fibroblast Growth Factor 21

Insulin resistance status was determined using the HOMA2 calculator released by the Diabetes Trials Unit, University of Oxford: (available at www.dtu.ox.ac.uk/homacalculator), which takes into account variations in tissue-specific IR, circulating proinsulin and variations in insulin secretion at FBG levels < 10mmol/L (Levy, 1998). The equation that the HOMA 2 is based on is as follows with %S denoting *insulin sensitivity*

R (which is the inverse of %S) = (insulin (pmol/L) X glucose (mmol/L))/22.5

However, for more accurate HOMA2-IR estimations it is recommended to use the calculator than to simply apply the equation (Levy, 1998).

### 3.4.6.2 Blood pressure

Blood pressure was measured three times using a sphygmomanometer (OMRON M2 Compact Upper Arm Digital Automatic Blood Pressure Monitor, OMRON HEALTHCARE CO., LTD, Kyoto, Japan) after participants were seated and been at rest for five minutes. From the three measurements, each obtained for systolic and diastolic blood pressure, a mean average value was calculated and used for the final analysis. Accuracy of the device is ± 3mmHg.

### 3.4.7 Secondary analysis of dietary intakes of carbohydrates and fatty acids

As reported in detail in chapter 3.4.1.2 ad-hoc dietary intake was assessed via four-day food diaries (FDs). For each participant in the EWG group the two time points were identified when CHO intake was at its highest and when it was at its lowest according to the FDs. For

each participant in the LCHF group the two FDs were identified when participants reported the highest and the lowest SFA intake. Therefore, 20 FDs were further analysed as follows. As even-chain fatty acids of different lengths seem to have differential effects from on CM risk for the LCHF arm the focus was on even-chain SFA with a length of between four and 18 carbons. The total amount of these SFAs reported in each FD was summed up and the percentage contribution of each individual even-chain SFA to total SFA intake was calculated. In addition, for each FD the percentage contribution of each food group (based on McCance and Widdowson food composition table nomenclature (Public Health England, 2019)) to the total amount of saturated fatty acid was calculated. For the EWG arm, the focus was on carbohydrate subclasses, namely starch, total sugars and fructose. The percentage contribution of each of these carbohydrate categories to total carbohydrate intake was calculated. In addition, the percentage contribution of each food group (based on McCance and Widdowson food composition table nomenclature (Public Health England, 2019)) to the total amount of carbohydrates consumed at either of the two time points by each individual participant was calculated.

### 3.4.8 Body composition

#### 3.4.8.1 Body composition

Body composition was assessed via 8-point stand-on bioelectrical impedance (BIA) at baseline, interim point and endpoint via seca mBCA 515 (seca®, Hamburg, Germany) according to manufacturer's instructions. The seca mBCA 515 proprietary software uses algorithms to quantity fat mass (FM) and VAT volume. Measurements were taken after a 12-hour overnight fast and following on from giving a venous blood sample. Participants were advised that they should drink water prior to attending the phlebotomy appointment but apart from this not consume any other food or drink. Bioelectrical impedance analysis in general, and the particular model use in the present study, has been validated against the gold standard methods, dual-energy x-ray absorptiometry (DXA) and magnetic resonance imaging (MRI) and found to be a valid method for measuring body composition in normal-weight and overweight adults, such as in the present study (Bosy-Westphal et al., 2017; Shafer et al., 2009).

#### 3.4.8..2 Anthropometrical assessment

Waist circumference (WC), and neck circumference (NC) were assessed at baseline, interim point and endpoint after a 12-our overnight fast using a tape measure calibrated in mm. Measurements were taken to the nearest mm and repeated twice according to the protocol applied in the NDNS RP 2008/09-2013/14 as described in chapter 3.3.3. The average of the three measurements was calculated. Neck circumference was measured according to PhenX

toolkit (consensus measures for PHENotypes and eXposures) Version 19 (www.phenxtoolkit.org, January 2017, Hamilton et al., 2011) just below the larynx perpendicular to the long axis of the neck. Participants were asked to hold their head straight, look ahead and keep their shoulders down. Three repeat measurements were taken to the nearest mm and the mean average of these calculated.

### 3.4.9 Physical activity

#### 3.4.9.1 Assessing pre-study moderate-to-vigorous physical activity levels

To assess habitual physical assessment prior to baseline measurements all study participants were asked to complete a Recent Physical Activity Questionnaire (RPAQ) (Appendix A) (see chapter 2.2.2) , which they received when they consented to take part and returned eight days prior to their baseline visit to the university laboratories. The RPAQ is a validated tool (Besson et al., 2010; Golubic et al., 2014) and a popular method to collect PA data as it is relatively quick and cheap to administer posing low participant burden (Panter et al., 2012). It is a subjective measure assessing habitual physical activity in four domains (home, work, commute and leisure) over the previous four-week period. Participants report both the frequency of and duration per occasion when they engaged in specific physical activities.

Each activity across the four domains has a MET score assigned based on Ainsworth et al.'s (2011) updated Compendium of Physical Activities to categorise the activity as sedentary, light, moderate or vigorous intensity. This approach is commonly used in research, including the NDNS RP (Mindell, 2014) (see chapter 2). Recoding of the completed RPAQ followed the script devised by MRC Epidemiology Unit (Scott et al., 2013; available to download at www.mrc-epid.cam.ac.uk/physical-activity-downloads/). First the total number of hours per day for each activity recorded in the four domains assessed (home, job, commute, leisure) over the previous 4-week period was calculated. The results from the individual domains were added to obtain average total daily duration (in hours) spent physically active. The data was then visually inspected, and time spent awake and engaged in physical activity was truncated at 18 hours. Where the 18-hour threshold was exceeded, data were adjusted as per MRC Epidemiology Unit script. Briefly, to begin with adjusted values were calculated for each variable that contributed to the total daily duration of time spent awake and engaged in physical activity. The time originally reported for each activity was multiplied by 18 and then divided by the total number of hours (awake and physically active) originally reported. After all the adjusted values for individual activities had been calculated, total number of hours per day spent awake and engaged in physical activity were re-calculated for all domains (see above). This adjustment had to be done for one of the participants in the LCHF

group. The total duration (hours/day) of all PA undertaken at moderate-to-vigorous intensity levels was calculated separately. In addition, one hour per day was allocated to each participant for personal hygiene (washing, getting dressed/undressed) (FAO, 2004). An allowance for sleep duration was truncated at 8 hours per day (Mindell, 2014).

#### 3.4.9.2 Objective assessments of moderate-to-vigorous physical activity levels

To obtain an objective measure of PA, participants were asked to wear a triaxial accelerometer (ActiGraph GT9X Link, ActiGraph LLC, Pensacola, FL) prior to each visit to LJMU laboratories at baseline, interim point and endpoint. The device was worn on participants' non-dominant wrist for seven consecutive days during waking hours, and participants were advised to wear the device for at least 10 hours each day. Data was collected at a sampling frequency of 30 Hz. Participants collected the device at least eight days prior to their baseline, interim point and endpoint visits respectively, and also received an information sheet confirming when and how to wear the device. In addition, they also completed a Wear time diary (Appendix A) to check compliance in wear time, validate the data recorded and account for any potential gaps in the data set. All participants were advised to not commence a new exercise regime whilst taking part in the study.

ActiGraph data were downloaded using ActiLife version 6.13.3 (ActiGraph LLC, Pensacola, FL), then saved in raw format as .gt3x files, and converted to .csv format for data processing. Data from the .csv files were processed using an open-source software R-package GGIR version 1.5-18 (http://cran.r-project.org). This package enables raw accelerometer data to be processed and analysed in R, including auto-calibration, detection of non-wear and calculation of the average magnitude of dynamic acceleration (Euclidean norm minus one [ENMO]) (Sabia et al., 2014; Van Hees et al., 2013; Van Hees et al., 2014). The outcome variables used for this intervention included wear time and MVPA time. Derived acceleration intensity thresholds (mg) for MVPA were set according to Hildebrand et al. (2014) as 100 (≥3MET). Files were excluded from the analysis if less than 10 hours of wear time were recorded in a calendar day. Minimum wear criteria of ≥10 hrs of wear on ≥3 days (weekday or weekend day) for each seven-day measurement period defined inclusion within analysis. Mean MVPA was retained for analysis to check whether participants met the government guidelines for PA for adults aged 19-64 of 150 min/week of MVPA (Department of Health, Physical Activity, Health Improvement and Protection, 2011) and to investigate any changes in MVPA. As one participant in the final analysis sample did not provide at least one valid weekend day at endpoint, data from an unweighted week were used for analysis and all days, regardless of being weekend days or weekdays, were treated equally.

### 3.5 Statistical Methods to analyse data collected

All statistical analysis was performed using IBM SPSS Statistics for Windows, Version 24.0 and version 25.0 (Armonk, NY: IBM Corp. Released 2016 and 2017 respectively).

#### 3.5.1 Dietary intake and food cravings

Average daily intake of total energy, CHO, total fat, SFA and of various food groups (the latter pre-study only) was analysed using a variety of statistical tests. Details of these can be found in Table 3.10. Descriptive statistics of plasma hydroxybutyrate concentrations were produced to assess presence of nutritional ketosis.

Normality for pre-study dietary intake and for differences between pre-study and baseline intake within or between groups was assessed via Shapiro-Wilk test ($p$>.05). In all ANOVAs presence of outliers was assessed by examination of studentized residuals for values greater than ±3. Normality was assessed by Q-Q Plot. Homogeneity of variances and co-variances ($p$>.05) was assessed by Levene's test of homogeneity and Box's M test respectively. Mauchly's test of sphericity was conducted to investigate whether the assumption of sphericity was retained in the two-way interaction ($p$ >.05).

Table 3.11 – Statistical tests undertaken to analyse dietary intake and food cravings of CALIBER participants

| Test | Purpose of analysis | Significance and effect size |
|---|---|---|
| Independent samples t-test | To assess group differences in habitual pre-study intake (normally distributed data) | $p$ <.05 |
| Mann-Whitney-U test | To assess group differences in habitual pre-study (non-normally distributed data where data could not be transformed) | $p$ <.05 |
| Paired samples t-tests | To assess differences between pre-study and baseline dietary intake for each group | $p$ <.05 |
| Two-way mixed ANOVA with diet as between-subjects factor and time as within-subject factor | To assess change in dietary intake over course of the study | $p$ <.05<br>Partial $\eta^2$ for effect size<br>≤.02 (small)<br>.02 – .13 (medium)<br>≥.14 (large) (Ellis, 2010) |
| Two-way mixed ANOVA with diet as between-subjects factor and time as within-subject factor | To assess change over course of study (mean score) in<br>Food cravings<br>How often yielded to cravings<br>How difficult to resist cravings | $p$ <.005<br>The Likert scale variables on FCI-UK are treated as continuous variables in analysis.<br>Although this is quite common practice, the more robust α has been used to account for the discussion surrounding the subject whether Likert scale variables should be treated as continuous or ordinal variables (Sullivan and Artino, 2013).<br>Partial $\eta^2$ for effect size<br>≤.02 (small)<br>.02 – .13 (medium)<br>≥.14 (large) (Ellis, 2010) |
| Paired samples t-test | Where two-way mixed ANOVA showed significant results for time or diet*time interaction.<br>To investigate within-samples differences<br>This was done because the post hoc function is not available on the two-way mixed ANOVA. | $p$ <.05 for dietary intake<br>$p$ <.005 for food cravings (see above) |
| Independent samples t-test | Where the two-way mixed ANOVA showed significant results for diet or diet*time interaction.<br>To investigate at which time points group differences were significant<br>This was done because the post hoc function is not available on the two-way mixed ANOVA. | $p$ <.05 for dietary intake<br>$p$ <.005 for food cravings (see above) |

### 3.5.2 Clinical markers of cardiometabolic risk

Linear mixed models for repeated measures with Maximum Likelihood Estimation were fitted to assess changes in HDL-C, LDL-C, sdLDL-C, TG, apoA1, apoB, TG/HDL-C ratio, apoB/apoA1 ratio, GLUC, INS, HOMA2, ADPN, LEPT, LAR, FGF21, DPB and SBP over the course of the study. The best-fit model for all variables (covariance type Compound Symmetry) contained diet group, time and diet*time-interaction as fixed factors. $p<.05$ was used as α. Visual inspection of the leptin data showed that the two female participants (one per diet arm) had considerably higher concentrations of leptin and data for one female participant (LCHF) was missing at baseline. The leptin data for the male sample was therefore analysed separately. Two participants (one in each study arm) had missing values for adiponectin at at least one time point. One participant in the EWG had unusually low systolic and diastolic blood pressure after the venous blood sample had been drawn at baseline. Their baseline data were not included in the analysis. The data for adiponectin, leptin (total sample) and for systolic and diastolic blood pressure were both analysed as *Intention to treat* (ITT) and as *per-protocol* (PP) to see whether there were any differences in findings. The results from the ITT were used in the decision-making process as to whether the intervention had any effects on the outcome variable to reduce the risk of bias and to maintain the principle of randomisation (Ranganathan et al., 2016). Linear mixed models, which were used to analyse the data, are robust and have the advantage of being able to deal with missing data better than repeated measures ANOVAs. Unlike ANOVA they can also model nonlinear data (Krueger and Tian, 2004).

### 3.5.3 Body composition

Linear mixed models for repeated measures with Maximum Likelihood Estimation were fitted to assess changes in FM (%), VAT (l), NC (cm) and WC (cm) over the course of the study. The best fit model for all FM (%), VAT (l), NC (cm) and WC (cm) (covariance type Compound Symmetry) contained diet group, time and diet-time-interaction as fixed factors. $P<.05$ was used as α.

### 3.5.4 Physical activity

A scatterplot showed that the relationship between the dependent variable and the covariate was not linear and therefore ANCOVA was not suitable as statistical method. For this reason, a linear mixed model was used for analysis. A linear mixed model with Maximum Likelihood Estimation was fitted to investigate group differences in habitual pre-study MVPA (hrs/day). Diet was used as a fixed factor, Participant ID was used as a random factor and baseline BMI ($kg/m^2$) was used as a co-variate. $P\leq.05$ was used as α.

Linear mixed models for repeated measures with Maximum Likelihood Estimation were fitted adjusted for daily accelerometer wear time (hrs) and BMI ($kg/m^2$) to assess changes in MVPA (min/d) over the course of the study. The best-fit model (covariance type Compound Symmetry: Heterogenous) contained diet group, time and diet*time interaction as fixed factors, BMI ($kg/m^2$) and daily accelerometer wear time (hours) as time-varying co-variates, and Participant ID as random effect (covariance type: AR(1)). $P \leq .05$ was used as $\alpha$.

A paired sample t-test for each intervention group was conducted to check for differences in pre-study and baseline MVPA (hrs/d) measured by self-report and by accelerometer respectively. $P \leq .05$ was used as $\alpha$.

## 3.6 Results

### 3.6.1 Dietary intake

#### 3.6.1.1 Pre-study habitual dietary intake

Based on the information provided by the participants in the FFQ the LCHF group consumed significantly more total fats and SFA, but only when intake was assessed in g/d (both *p*=.003) and not as %TE. They consumed non-significantly less CHO %TE but more CHO measured by weight. On average participants in the LCHF group consumed (non-significantly) >500 kcal/d more than in the EWG group. Of note is that the standard deviation for energy intake was considerably higher in the LCHF group (Table 3.12).

Table 3.12 – Comparison of habitual dietary intake of nutrients (M ± SD) between LCHF and EWG group over 12-month pre-study period

| | LCHF | EWG | p |
|---|---|---|---|
| **TE (kcal/d)** | 2534.9 ±557.0 | 2001.8 ±126.2 | 0.10 |
| **CHO%TE** | 40.8 ±4.4 | 42.6 ±6.2 | 0.61 |
| **Total CHO (g/d)** | 277.1 ±79.2 | 228.8 ±46.3 | 0.27 |
| **FAT%TE** | 38.4 ±4.6 | 35.8 ±4.9 | 0.41 |
| **Total fat (g/d)** | 107.8 ±22.7 | 78.6 ±7.7 | **0.03** |
| **SFA%TE** | 15.0 ±2.5 | 13.2 ±3.0 | 0.34 |
| **SFA (g/d)** | 42.0 ±9.3 | 28.9 ±5.8 | **0.03** |

CHO – carbohydrates; CHO%TE – carbohydrates percentage total energy; EWG= Eatwell Guide; FAT%TE – fat percentage total energy; g/d= grams per day; LCHF=low-carbohydrate, high-fat; SFA= saturated fatty acids; SFA%TE – saturated fatty acids percentage total energy; TE=Total energy

The analysis of the intakes of foods belonging to food groups that tend to be rich in CHO or rich in fats/SFA in the 12 months prior to study baseline only yielded significant differences for one food group. The LCHF group consumed on average significantly more meat and meat products than the EWG group (*p*=*.02*). For the majority of food groups analysed (Table 3.13) participants in the LCHF group consumed more of the foods, which is in accordance with the higher energy intakes reported above (Table 3.12).

Table 3.13 – Comparison of habitual dietary intake of food groups between LCHF and EWG group over 12-month pre-study period in grams per day (M ±SD or Median ±IQR)

| | LCHF | EWG | p |
|---|---|---|---|
| **CE* (g/d)** | 324.2 ±103.2 | 234.0 ±65.5 | 0.14 |
| **EG** (g/d)** | 21.5 ±19.5 | 32.0 ±44.8 | 0.91 |
| **FA* (g/d)** | 21.0 ±8.5 | 23.1 ±7.2 | 0.69 |
| **FR* (g/d)** | 241.2 ±236.4 | 352.7 ±197.7 | 0.44 |
| **ME* (g/d)** | 182.1 ±62.7 | 76.8 ±54.9 | **0.02** |
| **MI* (g/d)** | 503.8 ±147.5 | 558.0 ±87.7 | 0.50 |
| **NUT* (g/d)** | 19.0 ±12.4 | 14.1 ±12.3 | 0.54 |
| **POT* (g/d)** | 116.1 ±47.4 | 69.1 ±26.7 | 0.09 |
| **SUG* (g/d)** | 54.8 ±42.7 | 28.4 ±20.8 | 0.25 |
| **VEG* (g/d)** | 304.5 ±150.8 | 284.4 ±115.8 | 0.82 |

CE= Cereals and cereal products; EG= Eggs and egg dishes; FA= Fats and oils; FR= Fruit; EWG= Eatwell Guide; IQR=Inter-quartiles range; LCHF=low-carbohydrate, high-fat; ME= Meat and meat products; MI= Milk and milk products; NUT= Nuts and seeds; POT= Potatoes; SD=Standard deviation; SUG= Sugars; preserves and snacks; VEG= Vegetables *data analysed via Independent samples t-test and presented as M (±SD); ** data analysed via Mann-Whitney U test and presented as Median (±IQR)

### 3.6.1.2 Comparison between pre-study and baseline dietary intake

Compared to pre-study (assessed via FFQ) self-reported baseline (assessed via four-day FD) mean daily intake of SFA (%TE) was significantly lower in both the LCHF group ($p$=.037) and the EWG group ($p$=.012). Standard variations for total energy intake remained high in the LCHF group and had also considerably increased in the EWG group when food consumption was reported through a more immediate assessment method during the study (Table 3.14).

Table 3.14 – Self-reported dietary intake of total energy, carbohydrates, total fat and saturated fatty acids pre-study and at baseline (M ±SD)

| | LCHF | | | EWG | | |
|---|---|---|---|---|---|---|
| | **PRE** | **BL** | **p** | **PRE** | **BL** | **p** |
| **Total energy (kcal)** | 2534.9 ±557.0 | 2271.2 ±599.5 | .345 | 2001.8 ±126.2 | 2554.2 ±714.5 | .132 |
| **CHO (%TE)** | 40.8 ±4.4 | 40.2 ±9.6 | .91 | 42.6 ±6.2 | 43.6 ±9.4 | .673 |
| **CHO (g/d)** | 277.1 ±79.2 | 241.9 ±67.8 | .518 | 228.8 ±46.3 | 297.7 ±97.8 | .109 |
| **Total fats (%TE)** | 38.4 ±4.6 | 33.6 ±2.7 | .061 | 35.8 ±4.9 | 33.4 ±7.6 | .267 |
| **SFA (%TE)** | 15.0 ±2.5 | 11.6 ±1.8 | **.037** | 13.2 ±3.2 | 10.1 ±3.4 | **.012** |

%TE – percentage total energy, BL – Baseline (dietary intake assessed via four-day food diary), CHO – total carbohydrates, g/d – grams per day, EWG – Eatwell Guide, LCHF – Low-carbohydrate, high-fat, PRE – pre-study (dietary intake assessed via food frequency questionnaire), SFA – saturated fatty acids

### 3.6.1.3 Study-related ad-hoc dietary intake

As expected CHO intake in the LCHF group was significantly lower at interim point ($p$=.003 for %TE and $p$<.001 for g/d) and endpoint ($p$=.009 for %TE and $p$=.007 for g/d) compared to baseline. Intake of total fat was significantly higher ($p$=.019 for interim point and $p$=.001 for endpoint differences). Intake of CHO at these time points was also significantly lower than in the EWG group (all $p$<.005) and intake of and total fats was significantly higher ($p$=.012 for interim point and $p$<.0001 for endpoint differences). For all CHO (g/d), CHO (%TE) and total fat (%TE) significant effects for diet, time and diet-time interaction were observed (all $p$<.005). There were significant effects for diet ($p$=.006), time ($p$=*.003)* and diet-time interaction ($p$=.002) for SFA intake (%TE) but the differences between time points were not significant in either group. Intake of SFA doubled (albeit non-significantly) in the LCHF group but standard variations were also higher than at baseline. The EWG group consumed significantly less SFA at interim point and endpoint than the LCHF group. There were no significant differences and no significant interaction for total energy intake. Standard variations in both groups were considerable. (Table 3.15).

Table 3.15 – Daily total energy intake, carbohydrate, total fat and saturated fatty acid intake (Mean and standard deviation)

| | BL | IP | EP | Partial $\eta^2$ | p | F |
|---|---|---|---|---|---|---|
| **Total energy (kcal)** | | | | | | |
| **LCHF** | 2271.2 ±599.5 | 2056.8 ±328.7 | 1901.2 ±441.7 | Diet .129 Time .108 Diet*time .035 | Diet .309 Time .400 Diet*time .754 | Diet 1.182 Time 0.972 Diet*time 0.288 |
| **EWG** | 2554.2 ±714.5 | 2320.6 ±614.2 | 2431.0 ±921.2 | | | |
| **CHO (%TE)** †,‡‡, ## | | | | | | |
| **LCHF** | **40.2$^{\delta,\varepsilon}$ ±9.6** | **8.2$^{\delta}$ ±2.7** | **10.1$^{\varepsilon}$ ±5.6** | Diet .843 Time .708 Diet*time .702 | Diet **<.001** Time **<.001** Diet*time **<.001** | Diet 42.977 Time 19.357 Diet*time 18.879 |
| **EWG** | 43.6 ±9.4 | **44.6$^{\alpha}$ ±10.1** | **42.1$^{ß}$ ±7.8** | | | |
| **CHO (g)** †,‡‡, ## | | | | | | |
| **LCHF** | **241.9$^{\gamma,\varepsilon}$ ±67.8** | **44.2$^{\gamma}$ ±14.9** | **48.2$^{\varepsilon}$ ±22.1** | Diet .659 Time .630 Diet*time .507 | Diet **.004** Time **<.001** Diet*time **.003** | Diet 15.478 Time 13.645 Diet*time 8.229 |
| **EWG** | 297.7 ±97.8 | **267.7$^{ß}$ ±58.5** | **278.8$^{\alpha}$ ±142.0** | | | |
| **Fat (%TE)** †, ‡, ## | | | | | | |
| **LCHF** | **33.6$^{\gamma,\zeta}$ ±2.7** | **57.1$^{\gamma}$ ±15.2** | **60.5$^{\zeta}$ ±7.6** | Diet .642 Time .644 Diet*time .665 | Diet **<.005** Time **<.0001** Diet*time **<.0001** | Diet 14.349 Time 14.453 Diet*time 15.849 |
| **EWG** | 33.4 ±7.6 | **31.9$^{\alpha}$ ±8.3** | **33.5$^{ß}$ ±6.1** | | | |
| **SFA (%TE)** †, ‡, ## | | | | | | |
| **LCHF** | 11.6 ±1.8 | 23.3 ±8.7 | 22.3 ±5.1 | Diet .634 Time .515 Diet*time .538 | Diet .006 Time **.003** Diet*time **.002** | Diet 13.887 Time 8.491 Diet*time 9.321 |
| **EWG** | 10.1 ±3.4 | **9.3$^{\alpha}$ ±3.5** | **10.5$^{\alpha}$ ±2.8** | | | |

%TE – percentage total energy; BL – baseline; CHO – total carbohydrates; EP – endpoint; g – grams; EWG – Eatwell Guide; IP – interim point; LCHF – low-carbohydrate; high-fat; SFA – saturated fatty acids; F for diet*time (2,16); F for time (2,16): F for diet (1,8)

† diet*time interaction p<.005

‡ main effect of diet p<.05

‡‡ main effect of diet p<.005

# main effect of time p<.05

## main effect of time p<.005; for SFA (%TE) only post hoc tests did not show significant differences between time points for any group

$^{\alpha}$ p <.05 from LCHF at same time point

$^{ß}$ p <.005 from LCHF at same time point

$^{\gamma}$p<.05 between baseline and interim point in same group

$^{\delta}$p<.005 between baseline and interim point in same group

$^{\varepsilon}$p<.05 between baseline and endpoint point in same group

$^{\zeta}$p<.005 between baseline and endpoint point in same group

### 3.6.2 Adherence to diet

The four-day FDs completed at interim and endpoint showed that the EWG group derived on average 44.6% of their daily total energy from carbohydrates at interim point and 42.1% at endpoint, remaining below the target of 50% per day (Figure 3.5). The four-day FDs from the same time points for the LCHF group showed that mean daily intake of CHO remained within the 30 g – 50 g guidelines with mean intakes of 44.2g and 48.2g at interim and endpoint respectively.

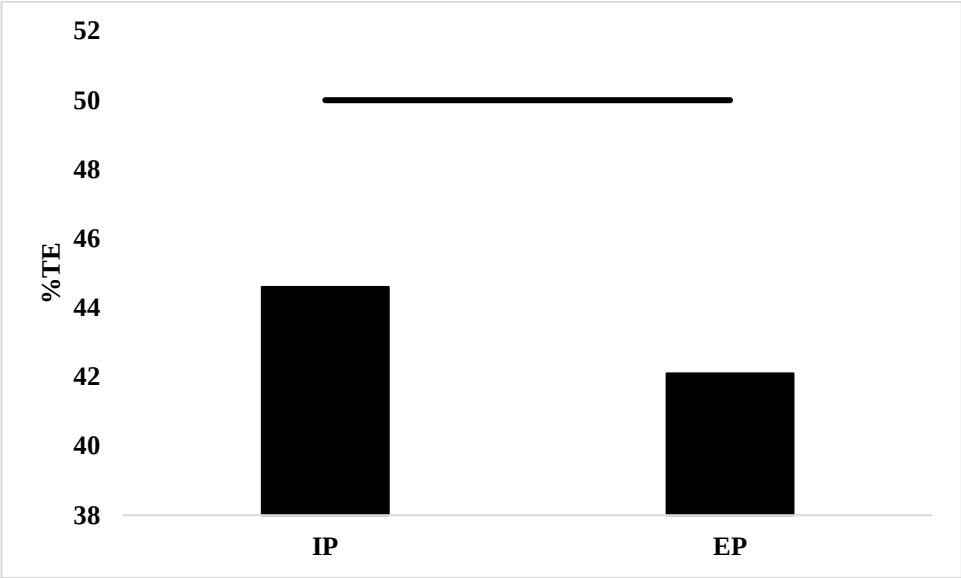


Figure 3.5 – Total energy derived (%) from carbohydrates in EWG group at interim point and endpoint compared to UK dietary guidelines
%TE – percentage total energy, EP – endpoint, IP – interim point

Mean plasma D3-hydroxybutyrate values in the LCHF group were below the 0.5 mmol/L threshold signifying nutritional ketosis (Harvey et al., 2018). At interim point, only two participants in the LCHF group were above the 0.5 mmol/L threshold with values of 0.8 mmol/L and 0.5 mmol/L respectively. At endpoint, none of the participants in the LCHF group were in nutritional ketosis with the highest value recorded as 0.3 mmol/L. None of the participants in the EWG group were at any time point in nutritional ketosis based on plasma D3-hydroxybutyrate levels (Table 3.16).

Table 3.16 – Plasma D3-hydroxybutyrate levels (M±SD) (mmol/L)

| | BL | IP | EP |
|---|---|---|---|
| **LCHF (mmol/L)** | .26±.00* | .36 ±.30 | .22 ±.11 |
| **EWG (mmol/L)** | <.07 | <.07 | <.07 |

BL – baseline; EP – endpoint; EWG – Eatwell Guide; IP – interim point; LCHF – low-carbohydrate, high-fat; *BL data for LCHF group based on one participant

### 3.6.3 Impact of dietary intake on food cravings

#### 3.6.3.1 Experience of food cravings

There were no significant differences between groups in the experience of food cravings over the course of the study, although the LCHF group decreased their cravings across all categories. The only significant effect observed was the effect of time for the cravings for fatty foods ($p$=.001) but post hoc tests did not show any significant differences between time points for either group. Cravings for starchy foods increased non-significantly in the EWG group but at the same time the standard deviations more than doubled (Table 3.17).

Table 3.17 –Scores for cravings of food overall, sugary, fatty, fast foods and starches (Mean ± SD)

| | BL | IP | EP | Partial $\eta^2$ | p* | F |
|---|---|---|---|---|---|---|
| **Overall** | | | | | | |
| **LCHF** | 2.88 ± 0.32 | 2.27 ±0.71 | 2.34 ±0.46 | Diet .396 Time .328 Diet*time .329 | Diet .051 Time .048 Diet*time .041 | Diet 5.251 Time 3.679 Diet*time 3.917 |
| **EWG** | 1.89 ± 0.32 | 1.90 ±0.47 | 1.90 ±0.43 | | | |
| **Sugary foods** | | | | | | |
| **LCHF** | 3.03 ±0.45 | 2.34 ±0.73 | 2.57 ±0.50 | Diet .410 Time .354 Diet*time.219 | Diet .046 Time .030 Diet*time .139 | Diet 5.555 Time 4.831 Diet*time 2.340 |
| **EWG** | 1.97 ±0.62 | 1.86 ±0.60 | 1.89 ±0.36 | | | |
| **Fatty foods** # | | | | | | |
| **LCHF** | 2.56 ±0.55 | 1.84 ±0.55 | 1.76 ±0.36 | Diet .209 Time .572 Diet*time .296 | Diet .184 Time **.001** Diet*time .060 | Diet 2.113 Time 10.684 Diet*time 3.360 |
| **EWG** | 1.84 ±0.46 | 1.60 ±0.49 | 1.64 ±0.17 | | | |
| **Fast foods** | | | | | | |
| **LCHF** | 2.72 ±0.44 | 2.60 ±0.60 | 2.68 ±0.46 | Diet .546 Time .009 Diet*time .098 | Diet .015 Time .932 Diet*time .438 | Diet 9.619 Time 0.071 Diet*time 0.871 |
| **EWG** | 1.60 ±0.57 | 1.76 ±0.54 | 1.72 ±0.58 | | | |
| **Starchy foods** | | | | | | |
| **LCHF** | 3.09 ±0.44 | 2.26 ±1.07 | 2.29 ±0.82 | Diet .074 Time .137 Diet*time .237 | Diet .449 Time .308 Diet*time .066 | Diet 0.635 Time 1.268 Diet*time 3.228 |
| **EWG** | 2.06 ±0.46 | 2.26 ±0.99 | 2.23 ±0.98 | | | |

BL – baseline, EP – endpoint, EWG – Eatwell Guide, IP – interim point, LCHF – low-carbohydrate, high-fat; F for diet*time(2.16); F for time (2,16); F for diet (1,8)

# main effect of time p<005; post hoc tests did not show any significant differences between time points for either group

*α<.005

### 3.6.3.2 Yielding to food cravings

There were no significant differences between groups when it came to yielding to cravings for any of the foods investigated, although significant diet-time interactions were observed for foods overall ($p$=.001) and starchy foods ($p$<.001). In the LCHF group participants reported that they between baseline and interim point and/or baseline and endpoint yielded significantly less to cravings for food overall and specifically for sugary and starchy foods (all $p$<.005). A significant effect of time could be observed for all groups but fast foods ($p$=.003 for fatty foods, remaining $p$<.001) (Table 3.18).

Table 3.18 –Mean scores yielding to cravings for food overall, sugary, fatty, fast foods and starchy foods (Mean ±SD)

| | BL | IP | EP | Partial $\eta^2$ | p | F |
|---|---|---|---|---|---|---|
| **Overall** †, # | | | | | | |
| **LCHF** | **2.63$^{\alpha}$ ±0.34** | **2.22$^{\alpha}$ ±0.54** | **1.64 ±0.25** | Diet .154 Time .711 Diet*time .561 | Diet .262 Time **<.0001** Diet*time **.001** | Diet 1.457 Time 19.698 Diet*time 10.216 |
| **EWG** | 1.79 ± 0.31 | 1.44 ±0.34 | 1.68 ±0.42 | | | |
| **Sugary foods** # | | | | | | |
| **LCHF** | **2.86$^{\alpha,ß}$ ±0.23** | **1.34$^{\alpha}$ ±0.46** | **1.49$^{ß}$ ±0.16** | Diet .052 Time .707 Diet*time .476 | Diet .525 Time **<.0001** Diet*time .006 | Diet .0.442 Time 19.266 Diet*time 7.267 |
| **EWG** | 1.97 ±0.72 | 1.60 ±0.64 | 1.66 ±0.37 | | | |
| **Fatty foods** #, ** | | | | | | |
| **LCHF** | 2.12 ±.072 | 1.12 ±0.18 | 1.40 ±0.37 | Diet .010 Time .516 Diet*time .165 | Diet .779 Time **.003** Diet*time .236 | Diet .0.084 Time 8.534 Diet*time 1.584 |
| **EWG** | 1.72 ±0.41 | 1.32 ±0.41 | 1.44 ±0.30 | | | |
| **Fast foods** | | | | | | |
| **LCHF** | 2.20 ±0.47 | 2.28 ±0.77 | 2.52 ±0.88 | Diet .476 Time .204 Diet*time .033 | Diet .027 Time .161 Diet*time .763 | Diet 7.278 Time 2.052 Diet*time 0.275 |
| **EWG** | 1.44 ±0.30 | 1.32 ±0.41 | 1.60 ±0.40 | | | |
| **Starchy foods*,** †, # | | | | | | |
| **LCHF** | **3.09$^{\alpha,ß}$ ±0.57** | **1.17$^{\alpha}$ ±0.19** | **1.32$^{ß}$ ±0.06** | Diet .004 Time .788 Diet*time .787 | Diet .861 Time **<.001** Diet*time **<.001** | Diet 0.032 Time$^{x}$ 29.772 Diet*time$^{x}$ 29.615 |
| **EWG** | 1.91 ±0.45 | 1.89 ±0.75 | 1.94 ±0.84 | | | |

BL – baseline, EP – endpoint, EWG – Eatwell Guide, IP – interim point, LCHF – low-carbohydrate, high-fat; F for diet*time(2.16); F for time (2,16); F for diet (1,8) with the exception of $^{X}$ F(1.191,9.563)
*Greenhouse-Geisser correction applied
** post hoc tests yielded no significance
† diet*time interaction p<005
# main effect of time p<.005
$^{\alpha}$ p<.005 between baseline and interim point in same group
$^{ß}$p<.005 between baseline and endpoint in same group

### 3.6.3.3 The difficulty to resist food cravings

Significant effects of time were observed for the difficulty to resist cravings overall (p<.0001) and for sugary (p=.001) and starchy foods (*p*<.0001). There was also significant diet-time interaction for starchy foods (*p*<.0001). The only effect that retained significance in post hoc tests was for the difficulty of resisting foods overall when the LCHF decreased these between baseline and interim point (*p*=.003) and baseline and endpoint (*p*=.001). Although non-significantly, the LCHF group reported considerably larger mean scores for nearly all categories. They also reported far less difficulties as the study progressed, whereas the EWG reported increased difficulties at endpoint to resist most food cravings assessed (Table 3.19).

Table 3.19 – Mean ± SD scores for how difficult it was to resist cravings for food overall, sugary, fatty, fast foods and starches

| | BL | IP | EP | Partial $\eta^2$ | p | F |
|---|---|---|---|---|---|---|
| **Overall** †, # | | | | | | |
| **LCHF** | **2.88$^{\alpha,ß}$ ±0.53** | **1.78$^{\alpha}$ ±0.81** | **2.14$^{ß}$ ±0.51** | Diet .172<br>Time .760<br>Diet*time .508 | Diet .433<br>Time **<.0001**<br>Diet*time **.003** | Diet 1.662<br>Time 25.343<br>Diet*time 8.264 |
| **EWG** | 1.99 ±0.41 | 1.62 ±0.39 | 1.98 ±0.46 | | | |
| **Sugary foods** #,** | | | | | | |
| **LCHF** | 3.11 ±0.76 | 2.06 ±0.99 | 2.54 ±0.67 | Diet .199<br>Time .572<br>Diet*time .154 | Diet .197<br>Time **.001**<br>Diet*time .261 | Diet 1.984<br>Time 10.689<br>Diet*time 1.462 |
| **EWG** | 2.26 ±0.47 | 1.77 ±0.75 | 2.97 ±0.51 | | | |
| **Fatty foods** | | | | | | |
| **LCHF** | 2.00 ±0.97 | 1.52 ±0.52 | 1.68 ±0.54 | Diet .010<br>Time .291<br>Diet*time .012 | Diet .782<br>Time .064<br>Diet*time .909 | Diet 0.082<br>Time 3.276<br>Diet*time 0.095 |
| **EWG** | 1.92 ±0.41 | 1.52 ±0.33 | 1.52 ±0.23 | | | |
| **Fast foods** | | | | | | |
| **LCHF** | 2.80 ±0.51 | 2.12 ±0.87 | 2.32 ±0.78 | Diet .329<br>Time .358<br>Diet*time .186 | Diet .083<br>Time .029<br>Diet*time .192 | Diet 3.915<br>Time 4.467<br>Diet*time 1.830 |
| **EWG** | 1.72 ±0.59 | 1.32 ±0.23 | 1.96 ±0.91 | | | |
| **Starchy foods** †, #,** | | | | | | |
| **LCHF** | 3.34 ±0.79 | 1.46 ±0.94 | 1.94 ±0.56 | Diet .042<br>Time .653<br>Diet*time .619 | Diet .572<br>Time **<.0001**<br>Diet*time **<.0001** | Diet 0.346<br>Time 15.040<br>Diet*time 12.975 |
| **EWG** | 1.97 ±0.65 | 1.75 ±0.55 | 2.31 ±0.79 | | | |

BL – baseline, EP – endpoint, EWG – Eatwell Guide, IP – interim point, LCHF – low-carbohydrate, high-fat; F for diet*time(2.16); F for time (2,16); F for diet (1,8)
† diet*time interaction p<005, # main effect of time p<.005
** post hoc tests yielded no significance
$^{\alpha}$ p<.005 between baseline and interim point in same group
$^{ß}$p<.005 between baseline and endpoint in same group

### 3.6.4 Adverse events

At interim point, all participants in the LCHF group reported a total of 14 adverse events. The most adverse event reported was general weakness (n=4) followed by headaches and flu-like symptoms (n=3), constipation (n=2) and impact on cognition/brain fog (n=2). At endpoint only three participants reported a total of four adverse events, which were bad breath (n=2), general weakness (n=1) and constipation (n=1).

### 3.6.5 Experience with the allocated diets

Overall, the practical implications for following their allocated diets seemed to have made more of a negative impact on the LCHF group in terms of socialising and the costs of the diet. All of the participants found it either “somewhat harder” or “a lot harder”, whereas as only one participant in the EWG group reported such difficulties. Three of the participants in the LCHF group also found the diet more expensive. Not everyone found it easy to follow a LCHF diet. In the home environment, LCHF participants seemed to have been more at ease with the allocated diet. The EWG group gave positive feedback regarding the practicalities of following the UK dietary guidelines and they did not feel that they had to adjust in their everyday life (Table 3.20).

Table 3.20 – Practical experiences with intervention in LCHF and EWG group

| | LCHF | EWG |
|---|---|---|
| **How easy or difficult to follow diet** | | |
| **Very easy** | 1 | 2 |
| **Somewhat easy** | 2 | 3 |
| **Not sure** | 1 | - |
| **Somewhat difficult** | 1 | - |
| **Very difficult** | - | - |
| **Shopping for ingredients** | | |
| **A lot easier** | 1 | - |
| **Somewhat easier** | - | - |
| **About the same** | 3 | 5 |
| **Somewhat harder** | 1 | - |
| **A lot harder** | - | - |
| **Cooking according to dietary rules** | | |
| **A lot easier** | 1 | - |
| **Somewhat easier** | - | - |
| **About the same** | 3 | 5 |
| **Somewhat harder** | 1 | - |
| **A lot harder** | - | - |
| **Costs of diet** | | |
| **A lot cheaper** | - | - |
| **Somewhat cheaper** | 1 | 1 |
| **About the same** | 1 | 4 |
| **Somewhat more expensive** | 3 | - |
| **A lot more expensive** | - | - |
| **Adhering to diet when socialising** | | |
| **A lot easier** | - | - |
| **Somewhat easier** | - | - |
| **About the same** | - | 4 |
| **Somewhat harder** | 3 | 1 |
| **A lot harder** | 2 | - |
| **Adhering to diet at home** | | |
| **A lot easier** | 1 | - |
| **Somewhat easier** | 1 | 1 |
| **About the same** | 3 | 4 |
| **Somewhat harder** | - | - |
| **A lot harder** | - | - |

EWG – Eatwell Guide, LCHF – low-carbohydrate, high-fat

Whilst there might have been some difficulties with the practical implications of following a LCHF diet, all participants in this group gave (very) positive feedback in terms of happiness, healthiness, alertness, quality of sleep and energy levels. The majority of EWG participants did not feel that following their allocated diet had improved their quality of life. Likewise, it had not had a negative affect either (Table 3.21).

Table 3.21 – Perceived impact on quality of life of intervention in LCHF and EWG group

| | LCHF | EWG |
|---|---|---|
| **Level of happiness** | | |
| **A lot happier** | 1 | - |
| **Somewhat happier** | 3 | 1 |
| **About the same** | 1 | 4 |
| **Level of healthiness** | | |
| **A lot healthier** | 3 | - |
| **Somewhat healthier** | 1 | 1 |
| **About the same** | 1 | 4 |
| **Level of alertness** | | |
| **A lot more alert** | 2 | - |
| **Somewhat more alert** | 3 | 1 |
| **About the same** | - | 4 |
| **Quality of sleep** | - | - |
| **Slept a lot better** | 2 | - |
| **Slept somewhat better** | 2 | 1 |
| **About the same** | 1 | 4 |
| **Energy levels** | | |
| **Feel a lot more energetic** | 2 | - |
| **Feel somewhat more energetic** | 3 | 2 |
| **About the same** | - | 3 |

EWG – Eatwell Guide; LCHF – low-carbohydrate, high-fat

All participants stated that they would continue to follow the allocated diet if their CM risk markers showed improvement. All study participants also felt that they had received sufficient support from the candidate over the course of the intervention. None of the participants used the Facebook group for social support as they stated that they had 'forgotten' that it was there and that they had not felt the need for additional online support as they could contact the candidate at any time.

### 3.6.6 Clinical markers of cardiometabolic risk

#### 3.6.6.1 Blood plasma marker

##### 3.6.6.1.1 Blood plasma lipids

###### 3.6.6.1.1.1 Plasma lipids overall results

There were significant findings for LDL-C, sdLDL-C and TG (Figure 3.6). A significant effect for diet ($p$=.038) and time ($p$=.048) could be observed for LDL-C concentrations. Throughout the intervention LDL-C concentrations were consistently higher in the LCHF than in the EWG group (Figure 3.6). Both groups had increased their LDL-C concentrations by endpoint. The overall increase was higher in the EWG group than in the LCHF group but concentrations remained lower than in the LCHF group (Figure 3.6). The LCHF group reached LDL-C peak concentrations at interim point, when CHO intake was reportedly at its lowest (chapter 3.6.1.3). There was a significant diet*time interaction ($p$=.008) for sdLDL-C concentrations (Figure 3.6). Whilst they were higher in the LCHF group than in the EWG group at baseline, at endpoint they were lower in the LCHF group. Small dense LDL-C concentrations decreased by 25% in the LCHF group and increased by 11% in the EWG

group. The decrease was continuous in the LCHF group but the increase in the EWG group occurred by interim point and stalled after this (Figure 3.6). The effect of time was found to be significant for TG concentrations ($p$=.035) (Figure 3.6). They were initially non-significantly higher in the LCHF group than in the EWG group. They decreased in both groups, but far more in the LCHF group (30% vs. 6%) (Figure 3.6). Whilst in the LCHF group the decline occurred by interim point and did not proceed further after this, the EWG group presented with the highest decline at interim point (19%) but TG concentrations increased again after this. They did however, remain below baseline levels (Figure 3.6). The effect of time was evident for the TG/HDL-C ratio ($p$=.033) (Figure 3.7). Whilst it was the same at baseline in both groups (albeit with a higher standard deviation in the EWG group), it decreased by 33% in the LCHF group, but only by 13% in the EWG group. The decrease in the TG/HDL-C ratio was continuous in the LCHF group. In the EWG group after a decrease that was bigger than in the LCHF group, the TG/HDL-C ratio increased again. It remained below baseline values (Figure 3.7).

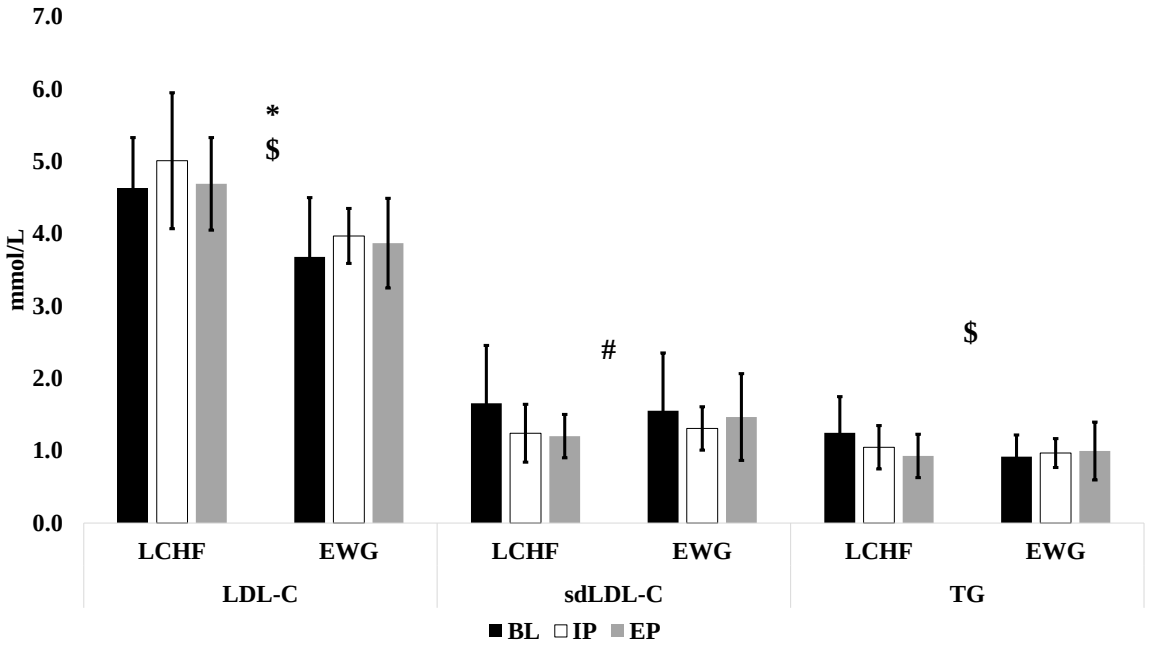


Figure 3.6 – Plasma concentrations (mmol/L) of low-density lipoprotein cholesterol, small dense low-density lipoprotein cholesterol and triglycerides at baseline, interim point and endpoint (Mean±SD)
BL – baseline; EP – endpoint; EWG – Eatwell Guide; IP – interim point; LCHF – low-carbohydrate, high-fat; LDL-C - low-density lipoprotein cholesterol, sdLDL-C – small dense low-density lipoprotein cholesterol; TG – triglycerides; *p<.05 for diet; $p<.05 for time; #p<.05 for diet*time; all data based on n=5 in LCHF group and n=5 in EWG group

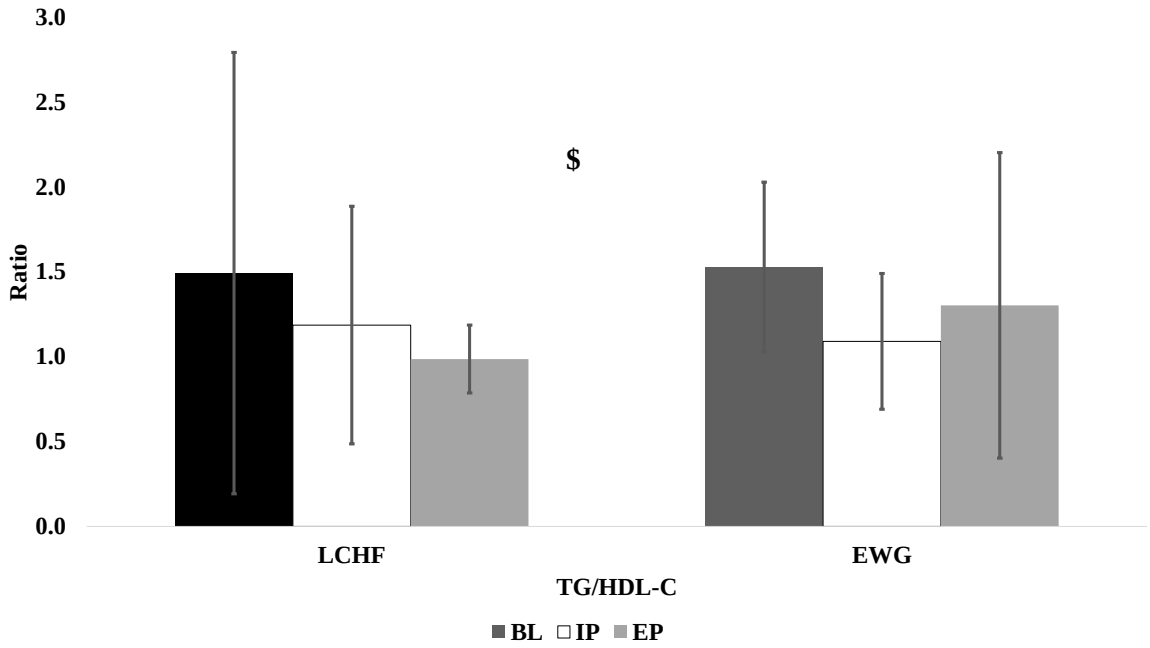


Figure 3.7 – Triglyceride/high-density lipoprotein cholesterol ratio at baseline, interim point and endpoint (Mean±SD)
BL – baseline; EP – endpoint; EWG – Eatwell Guide; IP – interim point; LCHF – low-carbohydrate, high-fat; TG/HDL-C - Triglyceride/high-density lipoprotein cholesterol ratio $p<.05 time; all data based on n=5 in LCHF group and n=5 in EWG group

There were no significant findings for HDL-C concentrations, which did not change at all in the EWG group and only slightly in the LCHF group (Table 3.22). There were no significant findings for apoA1 concentrations. These were nearly identical for both groups at baseline and increased for both. The increases were non-significantly higher in the EWG group (Table 3.22). There were also no significant findings for apoB. There was a slight decline in the LCHF group and an increase in the EWG group. Apolipoprotein B concentrations reached their peak at interim point in both groups (Table 3.22). The apoB/apoA1 ratio was higher in the LCHF group than the EWG group throughout the study. It decreased in the LCHF group and increased in the EWG group, although there was also an initial increase in the LCHF group. None of the results for the apoB/apoA1 ratio yielded significant findings (Table 3.22).

Table 3.22 – High-density lipoprotein and apolipoprotein B concentrations and apolipoproteinB/apolipoproteinA1 ratio at baseline, interim point and endpoint (Mean and standard deviation)

| | | LCHF (n=5) | EWG (n=5) | p* | F** |
|---|---|---|---|---|---|
| **HDL (mmol/L)** | **BL** | 1.1 ± 0.1 | 1.2 ±0.4 | Diet: .449 | 0.621 |
| | **IP** | 1.1 ± 0.2 | 1.3 ± 0.4 | Time: .140 | 2.172 |
| | **EP** | 1.2 ± 0.7 | 1.2 ± 0.4 | Diet*Time: .211 | 1.683 |
| **apoA1 (mg/dl)** | **BL** | 155.0 ± 9.1 | 154.8 ± 16.2 | Diet: .618 | 0.265 |
| | **IP** | 154.8 ± 16.2 | 165.2 ± 28.8 | Time: .240 | 1.534 |
| | **EP** | 159.6 ± 11.6 | 167.2 ± 32.0 | Diet*Time: .575 | 0.569 |
| **apoB (mg/dl)** | **BL** | 117.4 ± 16.2 | 101.0 ± 16.2 | Diet: .240 | 1.562 |
| | **IP** | 120.0 ± 24.9 | 111.2 ± 9.5 | Time: .373 | 1.036 |
| | **EP** | 114.8 ± 16.1 | 110.0 ± 14.3 | Diet*Time: .431 | 0.878 |
| **apoB/apoA1** | **BL** | 0.76 ± 0.13 | 0.66 ± 0.07 | Diet: .170 | 2.181 |
| | **IP** | 0.77 ± 0.11 | 0.68 ± 0.10 | Time: .464 | 0.799 |
| | **EP** | 0.72 ± 0.09 | 0.67 ± 0.14 | Diet*Time: .487 | 0.746 |

apoA1 – Apolipoprotein A-1; apoB – Apolipoprotein B; apoB/apoA1 – apolipoprotein B/apolipoprotein A-1 ratio; BL – baseline; EP – endpoint; EWG – Eatwell Guide; IP – Interim point; HDL – High-density lipoprotein cholesterol; LCHF – Low-carbohydrate, high-fat; *p values associated with type 3 tests of fixed effects ** Diet: F(1,10), Time:F(2,20), Diet*Time: F(2,20)

#### 3.6.6.1.1.2 Plasma lipids individual responses

In 40% of the LCHF arm participants LDL-C levels decreased between baseline and endpoint and in 60% of the participants they increased. In the EWG arm, LDL-C levels increased in 80% of the participants and only one participant presented with a decrease at intervention endpoint. Changes in LDL-C levels in the present sample were between -10% and +46% in the LCHF group and -34% and 17% in the EGW group (Figure 3.8).

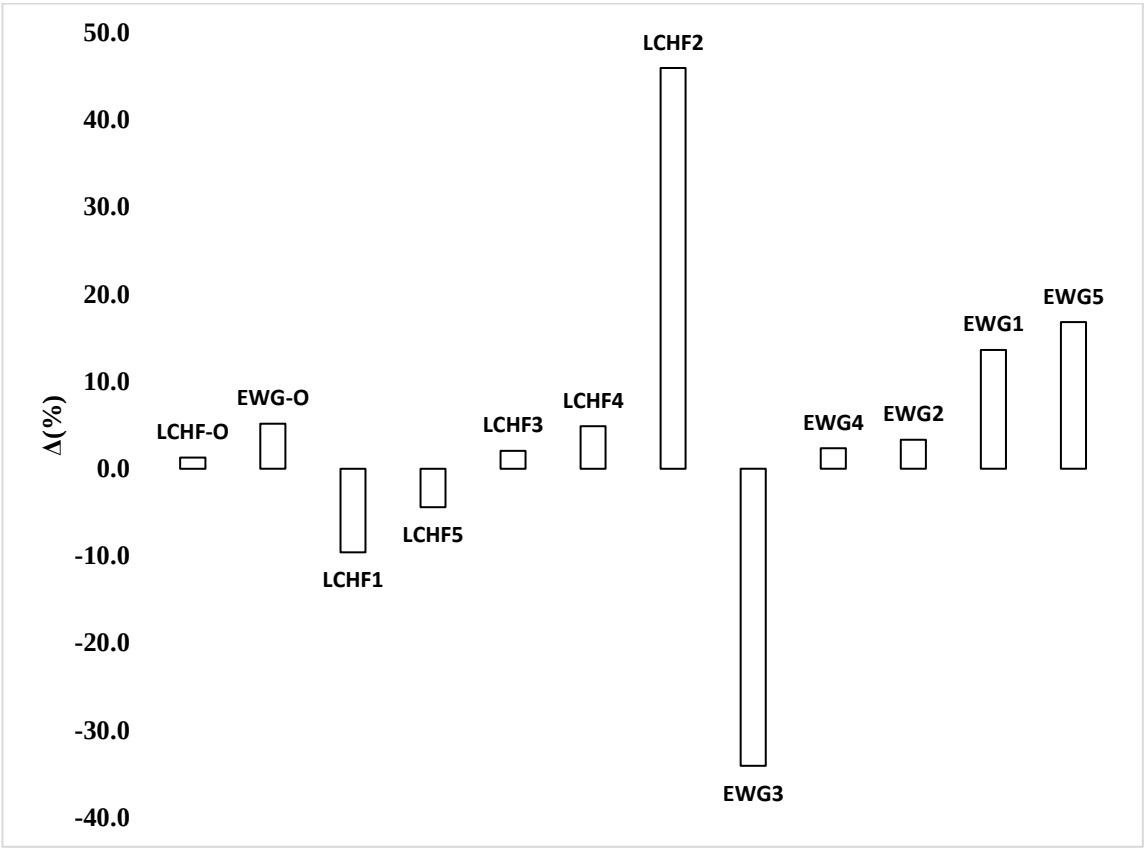


Figure 3.8 – Individual percentage changes to LDL-C concentrations from baseline to endpoint
EWG-O – Eatwell Guide overall group; LCHF-O – low-carbohydrate, high fat overall group; all data based on n=5 in LCHF group and n=5 in EWG group

In all the participants in the LCHF group sdLDL-C concentrations decreased by between 18% to 34%. In the EWG group only one participant presented with a decrease (by 17%), whereas the other subjects presented with increases by between 8 and 15% (Figure 3.9).

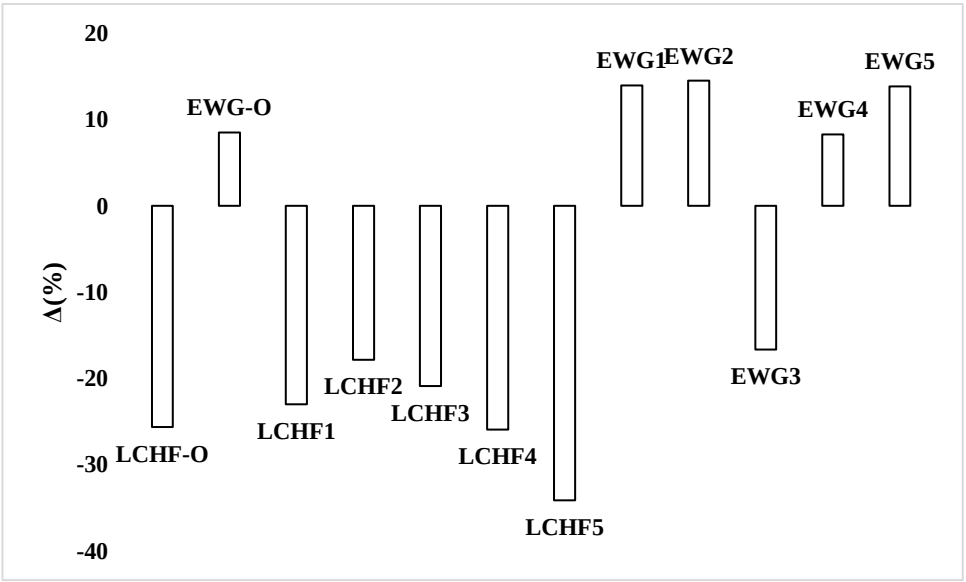


Figure 3.9 – Individual percentage changes to sdLDL-C concentrations from baseline to endpoint
EWG-O – Eatwell Guide overall group; LCHF-O – low-carbohydrate, high fat overall group; all data based on n=5 in LCHF group and n=5 in EWG group

Two participants in the LCHF arm and one participant in the EWG arm were hypertriglyceridaemic at baseline. At endpoint, the two subjects in the LCHF group had become normotriglyceridaemic. The subject in the EWG group presented with reduced TG concentrations but remained hypertriglyceridaemic. In 80% of participants in the LCHF, arm TG concentrations decreased by between 10% and 46% and in only one participant levels increased by 16%. In the EWG arm 40% of participants increased TG concentration by 10% and 11% respectively and 60% presented with decreased concentrations. The decreases ranged between 9% and 17% (Figure 3.10).

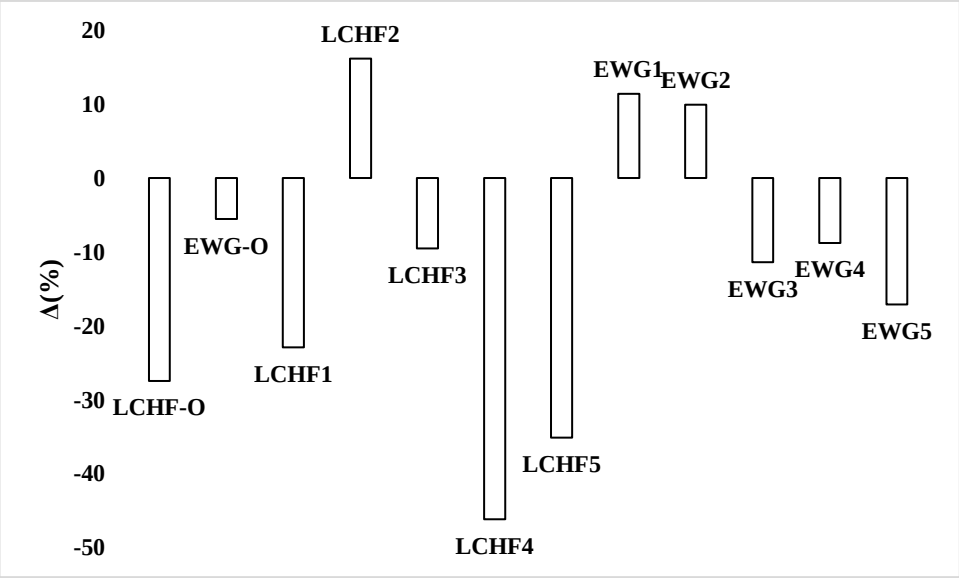


Figure 3.10 – Individual percentage changes to triglyceride concentrations from baseline to endpoint
EWG-O – Eatwell Guide overall group; LCHF-O – low-carbohydrate, high-fat overall group; all data based on n=5 in LCHF group and n=5 in EWG group

In the LCHF group four participants presented with decreased TG/HDL-C ratio (between -51% and -13%) and the ratio in one increased by 31%. In the EWG group three participants also presented with decreases in the TG/HDL-C ratio albeit the changes were not as large (ranging from -25% to -16%). Two participants in the EGW group had increases in the TG/HDL-C ratio by 1% and 5% respectively (Figure 3.11).

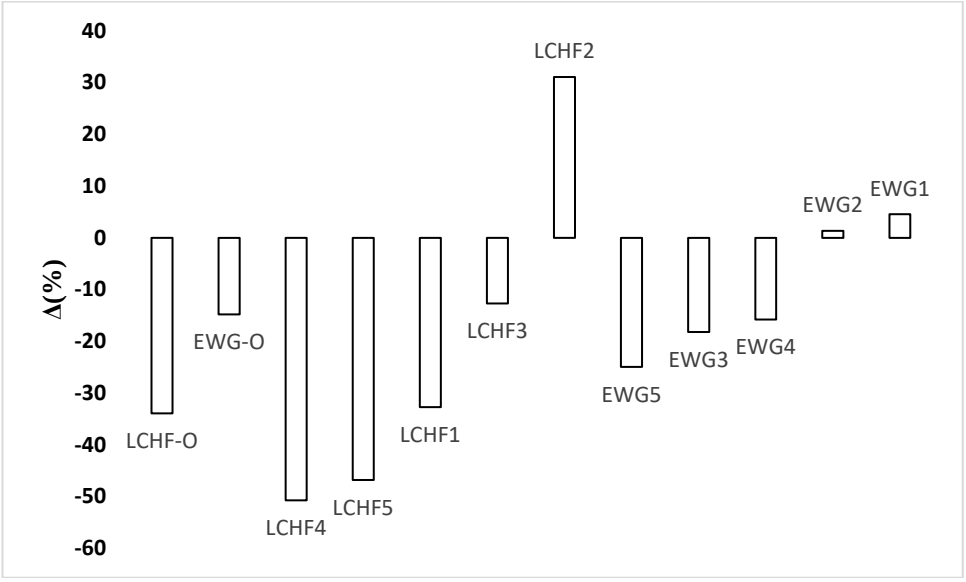


Figure 3.11 – Individual percentage changes to triglyceride/high-density lipoprotein ratio from baseline to endpoint
EWG-O – Eatwell Guide overall group; LCHF-O – low-carbohydrate, high-fat overall group; all data based on n=5 in LCHF group and n=5 in EWG group

#### 3.6.1.1.2 Glycaemic control and insulin resistance

##### 3.6.1.1.2.1 Glycaemic control and insulin resistance overall results

The effect of time was evident for insulin ($p$=.002) and HOMA2-IR ($p$=.003). Diet*time interaction was also observed for insulin ($p$=.035), and HOMA2-IR ($p$=.039). Fasting insulin was at baseline 26% higher in the LCHF than in the EWG group Insulin levels decreased in both groups, but more so in the LCHF group, where they reduced by 45% compared to a reduction in the EWG group by 12% (Figure 3.12). HOMA2-IR decreased in the LCHF group by 41% and by 8% in the EWG (Figure 3.13).

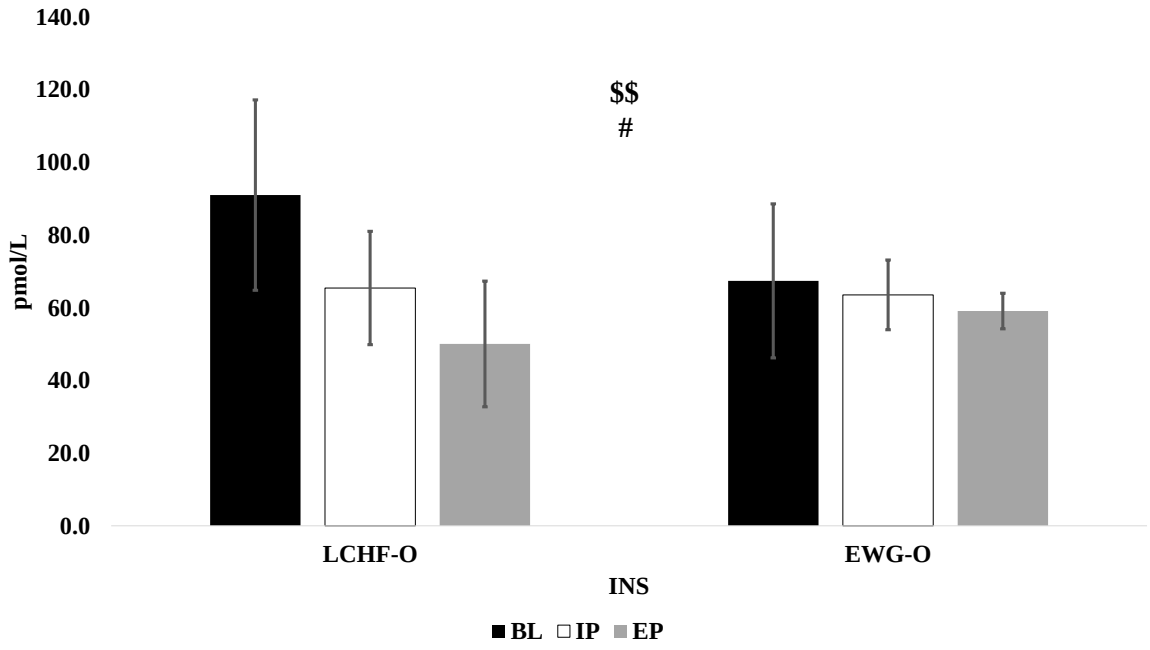


Figure 3.12 – Fasting insulin concentrations at baseline, interim point and endpoint (Mean±SD)
BL – baseline; EP – endpoint; EWG – Eatwell Guide; INS – insulin; IP – interim point; LCHF – low-carbohydrate, high-fat; $$p<.005 for time; #p<.05 for diet*time; all data based on n=5 in LCHF group and n=5 in EWG group

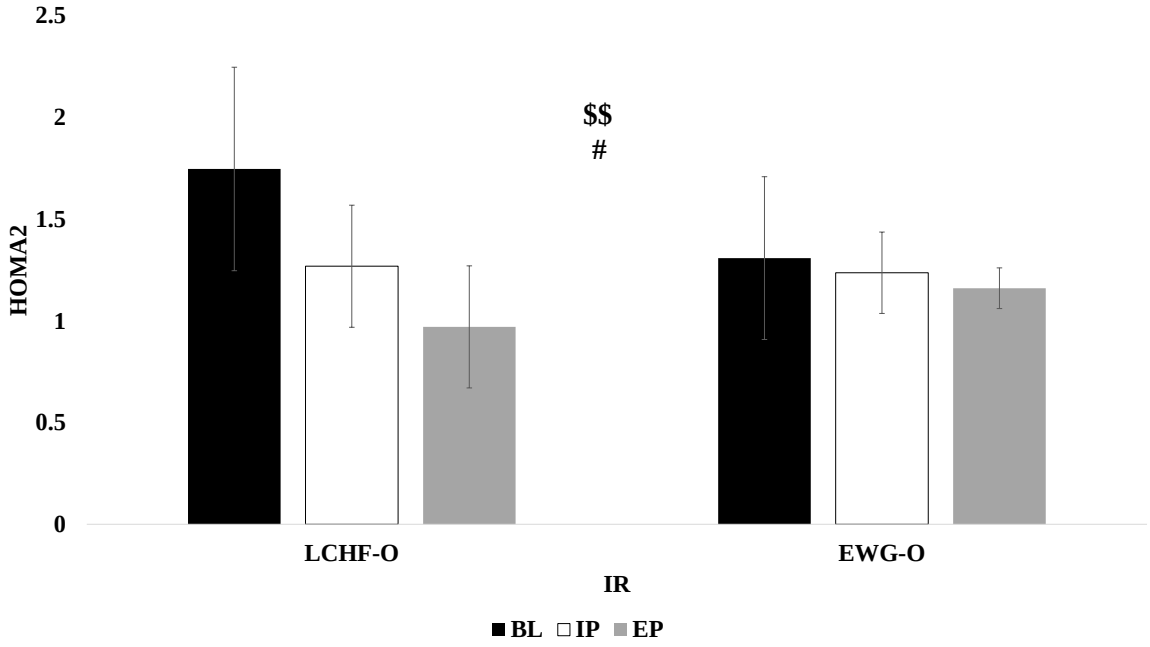


Figure 3.13 – Insulin resistance at baseline, interim point and endpoint (Mean±SD)
BL – baseline; EP – endpoint; EWG – Eatwell Guide; IP – interim point; IR – insulin resistance; LCHF – low-carbohydrate, high-fat; $$p<.005 for time; #p<.05 for diet*time; all data based on n=5 in LCHF group and n=5 in EWG group

There were no significant changes in FPG concentrations in either group. Concentrations were similar between groups and did not change over time either (Table 3.23).

Table 3.23 – Fasting plasma glucose concentrations at baseline, interim point and endpoint (Mean±SD)

| | | LCHF (n=5) | EWG (n=5) | p* | F** |
|---|---|---|---|---|---|
| **GLUC (mmol/L)** | **BL** | 5.8 ± 0.5 | 6.0 ± 0.3 | Diet: .365 | 0.900 |
| | **IP** | 5.9 ± 0.4 | 6.0 ± 0.3 | Time: .709 | 0.350 |
| | **EP** | 5.8 ± 0.4 | 6.0 ± 0.4 | Diet*Time: .243 | 1.518 |

BL – baseline; EP – endpoint; EWG – Eatwell Guide; GLUC – Fasting plasma glucose; IP – Interim point; LCHF – Low-carbohydrate, high-fat; *p values associated with type 3 tests of fixed effects ** Diet: F(1,10), Time: F(2,20), Diet*Time: F(2,20)

### 3.6.1.1.2.2 Glycaemic control and insulin resistance individual responses

After 8 weeks, all participants in the LCHF arm decreased their fasting plasma insulin concentration by between 15% and 59% and their HOMA2-IR by between 14% and 59%. In the EGW arm 60% of participants presented with decreased insulin concentrations (ranging from 11% to 35%) and with decreased HOMA2-IR (ranging from 10% to 35%). In the same group 40% presented with insulin increases of 24% and 28% and HOMA2-IR increases of 26% and 29% (Figure 3.14 and Figure 3.15).

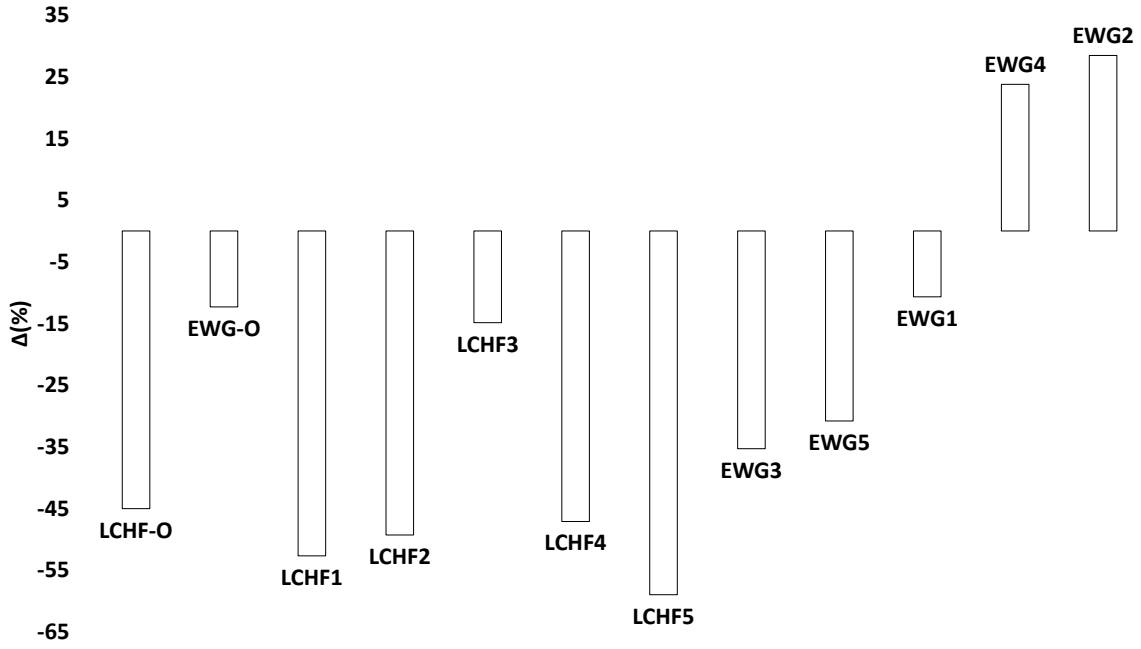


Figure 3.14 – Individual percentage changes in insulin concentrations from baseline to endpoint
EWG-O – Eatwell Guide overall group; LCHF-O – low-carbohydrate, high-fat overall group; all data based on n=5 in LCHF group and n=5 in EWG group

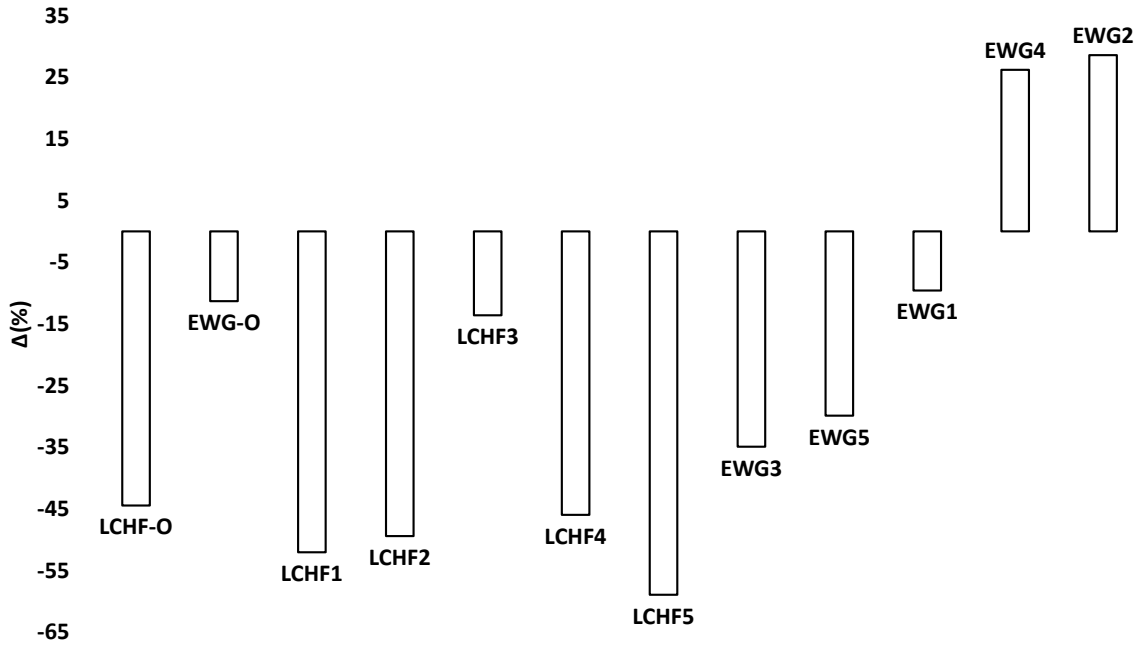


Figure 3.15 – Individual percentage changes in insulin resistance (HOMA2) from baseline to endpoint
EWG-O – Eatwell Guide overall group; LCHF-O – low-carbohydrate, high-fat overall group; all data based on n=5 in LCHF group and n=5 in EWG group

### 3.6.6.1.3 Adipokines

#### 3.6.6.1.3.1 Adipokines overall results

There was a significant diet*time interaction for adiponectin ($p$=.028). Throughout the intervention concentrations were lower in the LCHF group than in the EWG group. The difference was as high as 223% (at interim point). However, the standard deviations were very high in the EWG arm. The PP analysis revealed that after exclusion of the two participants with missing data the diet*time interaction was no longer significant ($p$=.052) (data not shown). Adiponectin concentrations decreased in the LCHF group over the course of the intervention by 4%. Concentrations in the EWG arm increased between baseline and endpoint. Highest concentrations of adiponectin were reached in the EWG arm at interim point (Figure 3.16).

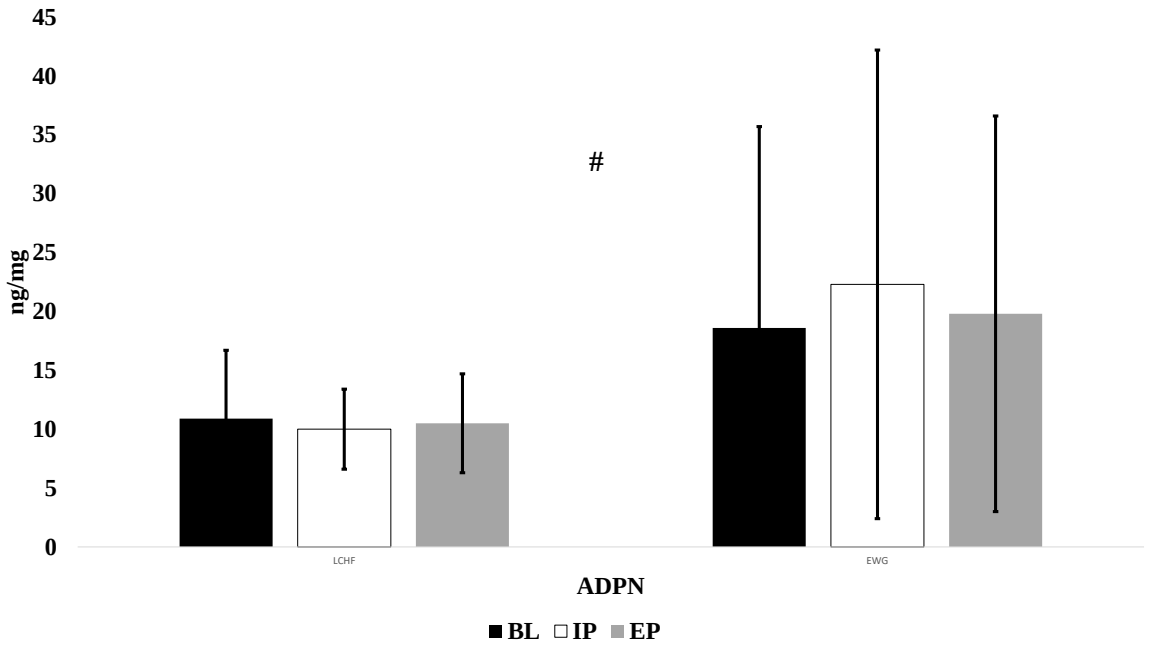


Figure 3.16 – Adiponectin concentrations at baseline, interim point and endpoint (Mean±SD)
ADPN – adiponectin; BL – baseline; EP – endpoint; EWG – Eatwell Guide; IP – interim point; LCHF – low-carbohydrate, high-fat; #p<.05 for diet*time; results from the intention to treat analysis; all data based on n=5 in LCHF group and n=5 in EWG group

There were no significant changes for leptin or the leptin/adiponectin ratio (LAR) for the total sample. Standard deviations for both markers were high (Table 3.24). In the per-protocol analysis there was a significant diet*time interaction ($p$=.002) (data not shown). Analysis of the data for the male participants only resulted in lower mean values and much lower standard deviation. There were significant differences for diet ($p$=.001) and the effect of time and diet*time interaction was also significant (both $p$<.001) in males. There were no significant findings for the LAR for the males only sample either (Table 3.24). At baseline and interim point leptin concentrations were higher in the LCHF group than in the EWG group. At endpoint they were the same. In the LCHF group leptin concentrations decreased, whilst they increased in the EWG group. The sharpest decline in leptin for the LCHF group (males only) occurred between baseline and interim point (Figure 3.17).

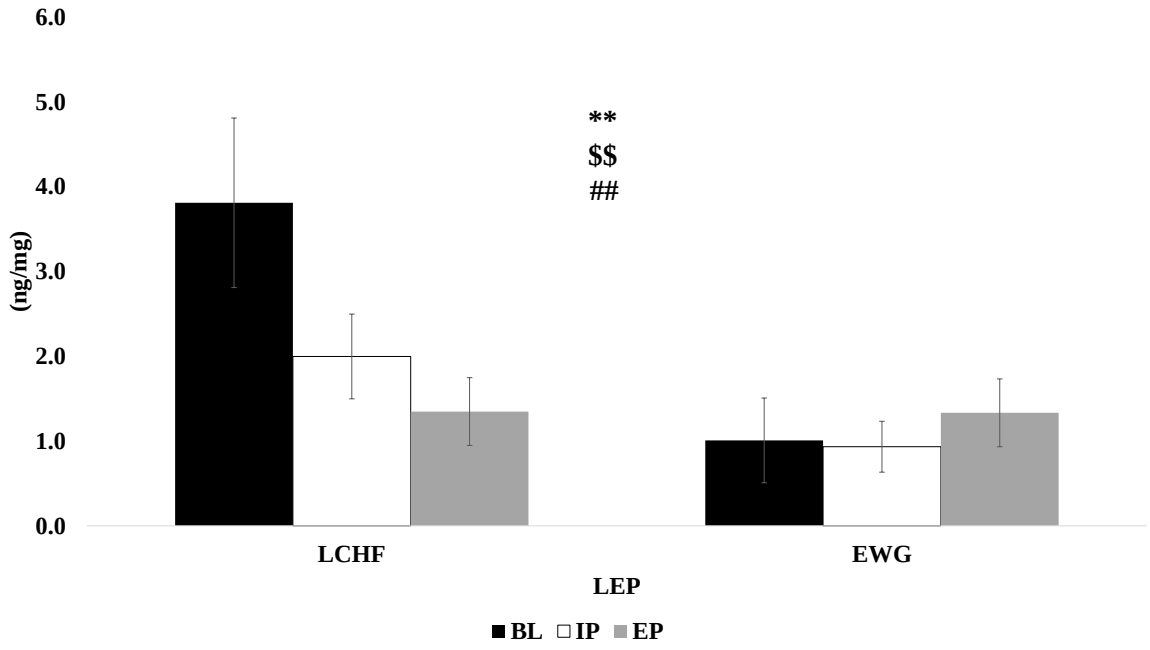


Figure 3.17 – Leptin concentrations at baseline, interim point and endpoint in male participants only (Mean±SD)
BL – baseline; EP – endpoint; EWG – Eatwell Guide; IP – interim point; LCHF – low-carbohydrate, high-fat; **p<.005 for diet; $$p<.005 for time; ##p<.005 for diet*time; results from the intention to treat analysis
Data shown for eight participants (n=4 in LCHF group and n=4 in EWG group)

Table 3.24 –Leptin concentrations and the leptin/adiponectin ratio at baseline, interim point and endpoint (Mean±SD)

| | | LCHF | EWG | p**** | F***** |
|---|---|---|---|---|---|
| **LEPT* (ng/mg)** | **BL** | 3.8 ± 1.0 | 4.5 ± 7.8 | Diet: .625 | 0.254 |
| | **IP** | 6.7 ± 10.5 | 4.5 ± 8.0 | Time: .166 | 1.976 |
| | **EP** | 8.2 ± 15.3 | 5.9 ± 10.3 | Diet*Time: .217 | 1.659 |
| **LAR**** | **BL** | 0.5 ± 0.3 | 0.3 ± 0.6 | Diet: .380 | 0.843 |
| | **IP** | 0.7 ± 1.0 | 0.3 ± 0.6 | Time: .150 | 2.109 |
| | **EP** | 0.8 ± 1.5 | 0.4 ± 0.7 | Diet*Time: .439 | 0.861 |
| **LAR***** | **BL** | 0.3 ± 0.5 | 0.7 ± 0.4 | Diet: .147 | 2.584 |
| | **IP** | 0.2 ± 0.1 | 0.0[a] ± 0.1 | Time: .773 | 0.261 |
| | **EP** | 0.2 ± 0.3 | 0.0[b] ± 0.1 | Diet*Time: .761 | 0.278 |

BL – baseline; EP – endpoint; EWG – Eatwell Guide; IP – Interim point; LAR – leptin/adiponectin ratio; LEPT – leptin; LCHF – Low-carbohydrate, high-fat; [a]value=0.0140; [b]value=0.0461
*analysis total sample (n=5 in LCHF group and n=5 in EWG group; data missing for n=1 at BL in LHCF group) (ITT)
**analysis total sample (n=5 in LCHF group and n=5 in EWG group; data missing for n=1 at BL in LHCF group) (ITT)
***analysis male sample only (n=4 in LCHF group and n=4 in EWG group) (ITT)
****p values associated with type 3 tests of fixed effects
*****for LEPT*: Diet: F(1,9.934), Time: (2,18.944), Diet*Time: F(2,18.944); for LAR**: Diet: F(1,9.91), Time: F(2,17.937), Diet*Time: F(2,17.937); for LAR***: Diet: F(1,8), Time: F(2,16), Diet*Time: F(2,16);

### 3.6.6.1.3.2 Adipokines individual responses

In the LCHF arm 75% of participants with complete data presented with decreased adiponectin concentrations at endpoint with reductions ranging from 4% to 11%. The remaining participant presented with an increase of 14%. In the EGW arm 75% of participants with complete data presented with increased adiponectin concentrations at endpoint with increases ranging from 4% to 23%. The remaining participant presented with aa decrease of 8% (Figure 3.18).

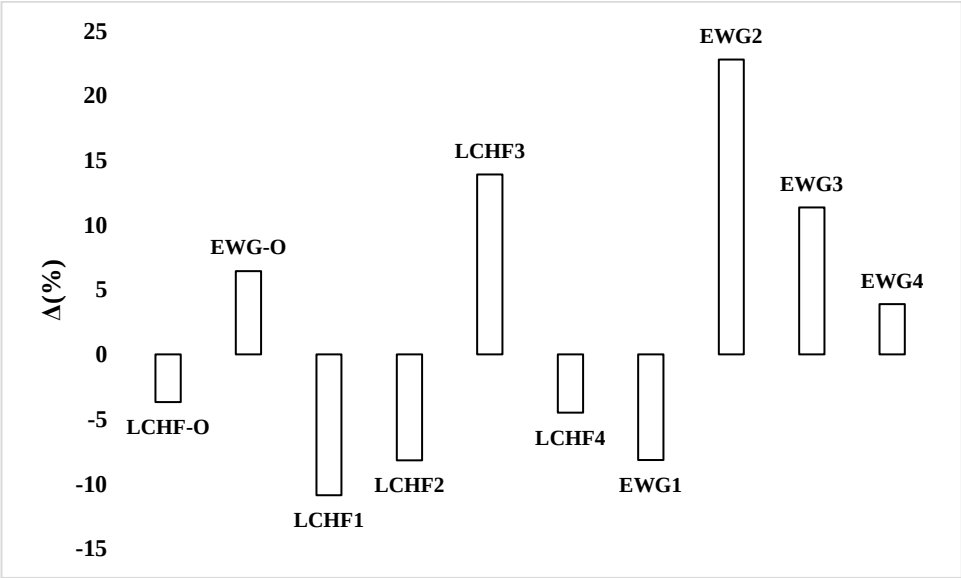


Figure 3.18 – Individual percentage changes in adiponectin concentrations from baseline to endpoint
EWG-O – Eatwell Guide overall group; LCHF-O – low-carbohydrate, high-fat overall group
Data presented for eight participants (n=4 in LCHF group and n=4 in EWG group)

All male participants of the LCHF arm decreased their leptin concentrations by between 59% and 70%, whereas in the EWG arm three male participants increased their concentrations by between 2% and 243% and one male participant decreased their leptin concentrations by 30% (Figure 3.19).

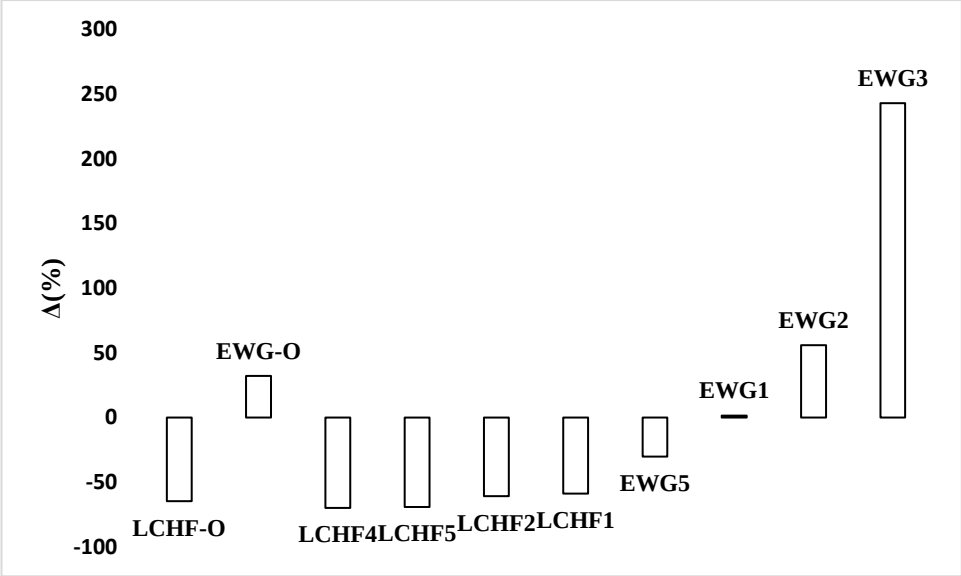


Figure 3.19 – Individual percentage changes in leptin concentration in men from baseline to endpoint
EWG-O – Eatwell Guide overall group; LCHF-O – low-carbohydrate, high-fat overall group
Data presented for eight (male) participants (n=4 in LCHF group and n=4 in EWG group)

### 3.6.6.1.4 Hepatokine

#### 3.6.6.1.4.1 Hepatokine overall results

The effect of time was evident for FGF21 concentrations ($p$=.044). They were higher in the EWG group than in the LCHF group throughout the intervention. They declined in the LCHF group overall, but steadily increased in the EWG group. Concentrations nearly halved in the LCHF group between baseline and interim point, then increased again but remained nearly 18% below baseline levels. Between baseline and endpoint FGF21 concentrations increased by 65% in the EWG group (Figure 3.20).

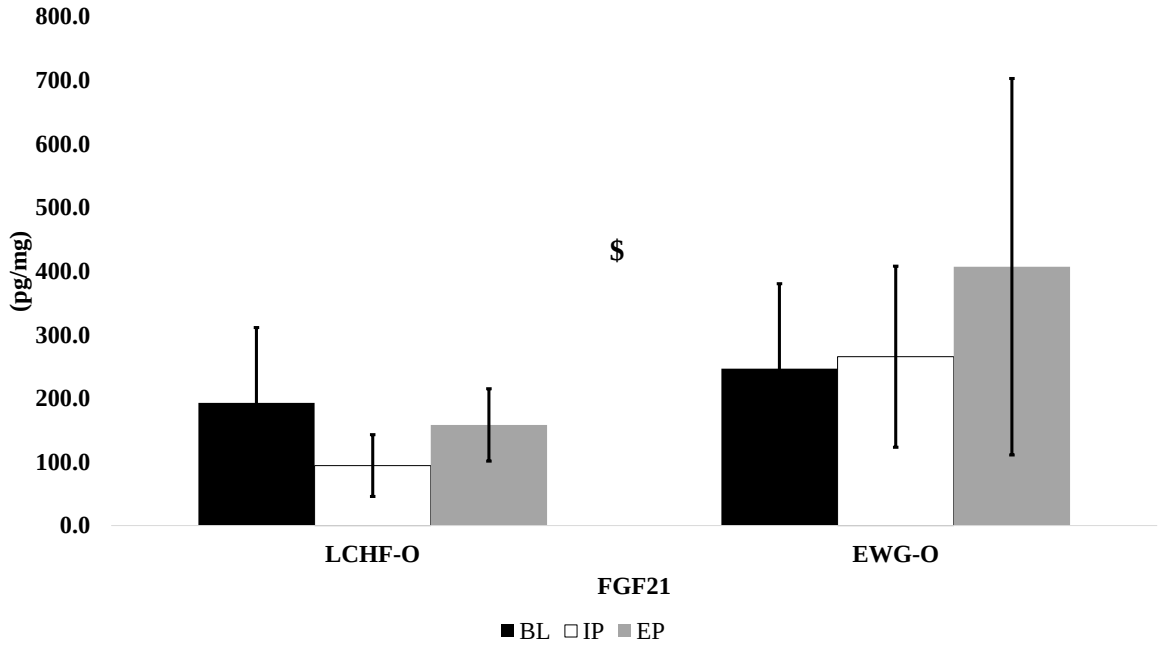


Figure 3.20 – Fibroblast growth factor 21 concentrations at baseline, interim point and endpoint (Mean±SD) BL – baseline; EP – endpoint; EWG – Eatwell Guide; FGF21 – fibroblast growth factor 21; IP – interim point; IR – insulin resistance; LCHF – low-carbohydrate, high-fat; $p<.05 for time; all data based on n=5 in LCHF group and n=5 in EWG group

### 3.4.3.4.2 Hepatokine individual responses

Eighty percent of participants in the LCHF group presented with a decrease of FGF21 concentrations of between 3% and 54%. However, one participant presented with an increase of 197% at endpoint. In the EWG group 80% of participants also presented with an increase at endpoint with changes ranging from 33% to 116%, whereas one participant presented with a 3% decrease in concentrations (Figure 3.21).

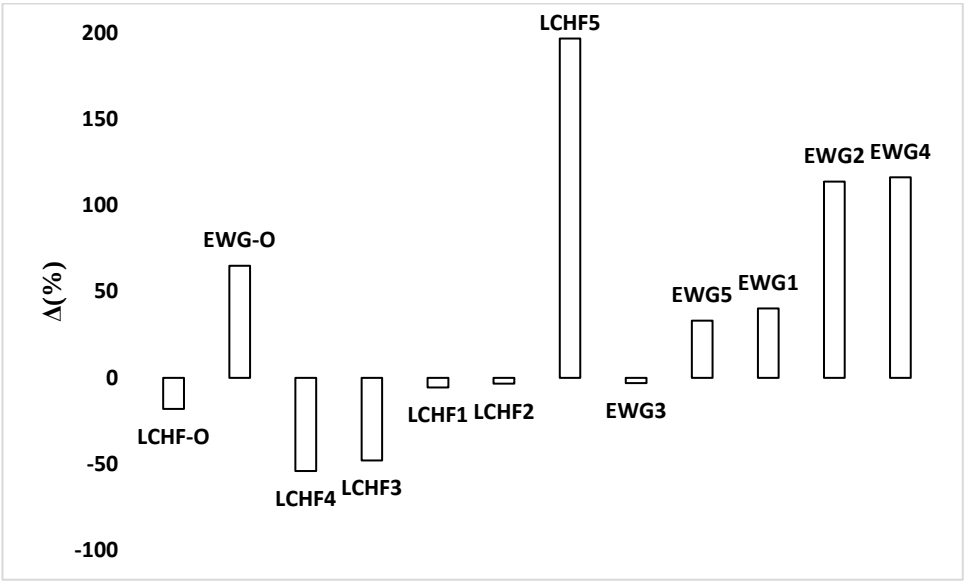


Figure 3.21 – Individual percentage changes in fibroblast growth factor 21 concentrations from baseline to endpoint
EWG-O – Eatwell Guide overall group; LCHF-O – low-carbohydrate, high-fat overall group; all data based on n=5 in LCHF group and n=5 in EWG group

### 3.6.6.2 Systolic and diastolic blood pressure

#### 3.6.6.2.1 Systolic and diastolic blood pressure overall results

There was diet*time interaction for SBP ($p$=.005) and the effect of time was significant for DBP ($p$=.020) in the ITT analysis. Both SBP and DBP were similar in the two groups at baseline. Only DBP decreased in the EWG group (by 3%), SBP did not change. Both decreased in the LCHF group. Systolic blood pressure decreased by 7% and DBP decreased by 10% in the LCHF group over the course of the intervention. (Figure 3.22). In the PP analysis there was a significant effect for diet ($p$=.044) and diet*time interaction ($p$=.019) for SBP and a significant effect for time and diet*time interaction (both $p$=.038) for DBP (data not shown).

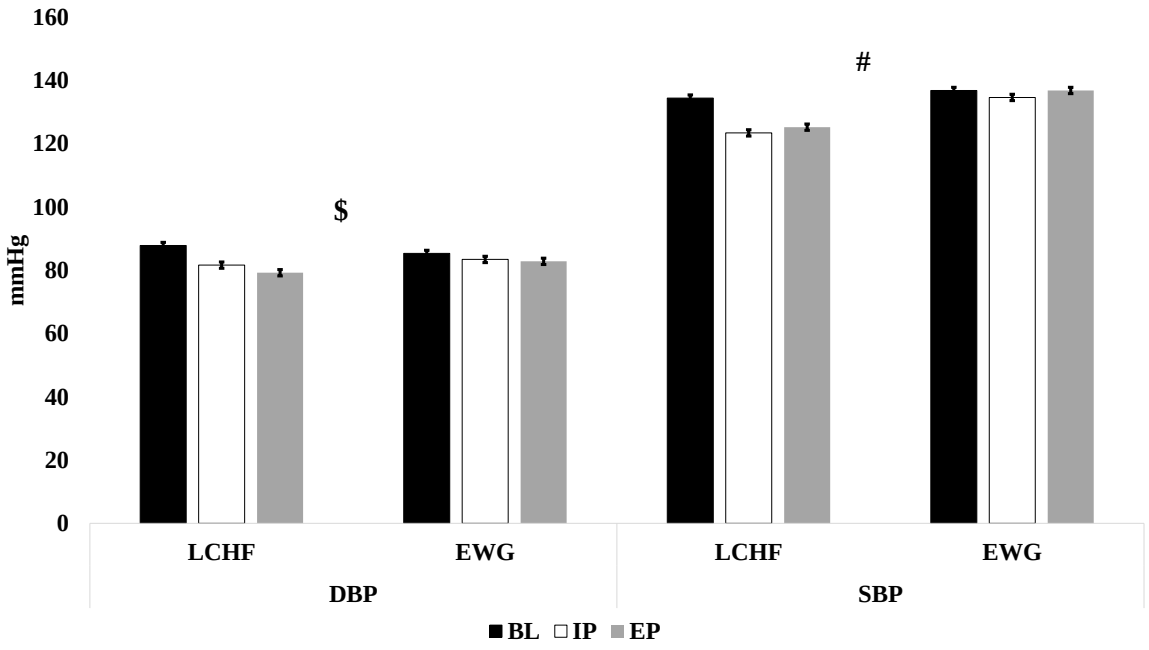


Figure 3.22 – Systolic and diastolic blood pressure at baseline, interim point and endpoint (Mean±SD)
BL – baseline; DBP – diastolic blood pressure; EP – endpoint; EWG – Eatwell Guide; IP – interim point; IR – insulin resistance; LCHF – low-carbohydrate, high-fat; SBP – systolic blood pressure; $p<.05 for time; #p<.05 for diet*time; results shown from intention to treat analysis
Baseline data based on nine participants (n=5 in LCHF group and n=4 in EWG group)

#### 3.6.6.1.2 Systolic and diastolic blood pressure individual responses

All participants in the LCHF arm presented with decreases in SBP at endpoint ranging from 6% to 11%. In the EWG arm only one participant presented with a decrease of 4%, whereas the remaining participants in this group presented with increases of between 2% and 6% (Figure 3.23). All participants in the LCHF arm also presented with decreases in DBP ranging from 6% to 15%. In the EWG arm the same participant presented with a decrease of 9% in DBP, whereas the remaining participants in this group presented with increases of between 1% and 5% (Figure 3.24).

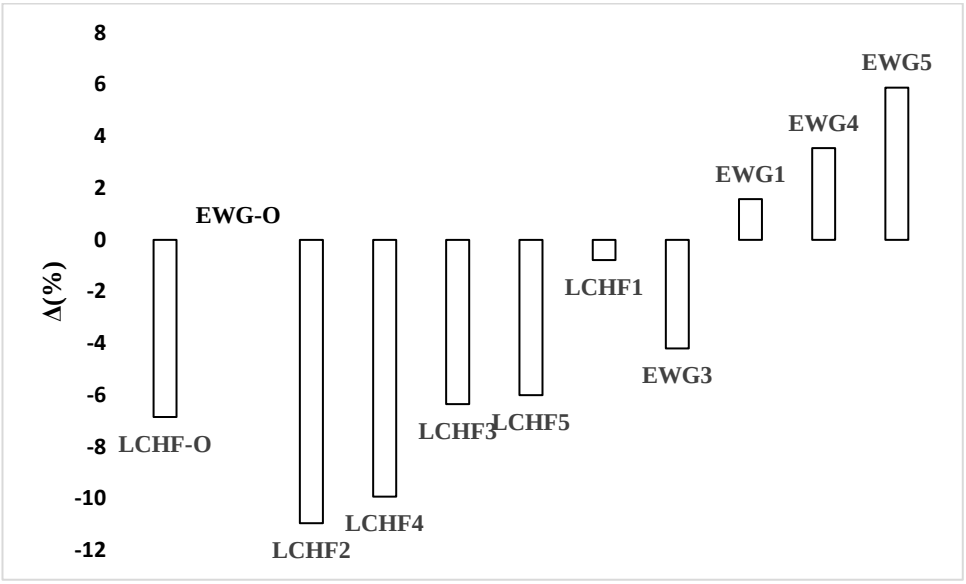


Figure 3.23 – Individual percentage changes in systolic blood pressure from baseline to endpoint
EWG-O – Eatwell Guide overall group; LCHF-O – low-carbohydrate, high-fat overall group
Data for participant EWG2 not shown due to extremely low SBP measurement at baseline; Δ% for EWG-O *=0.*

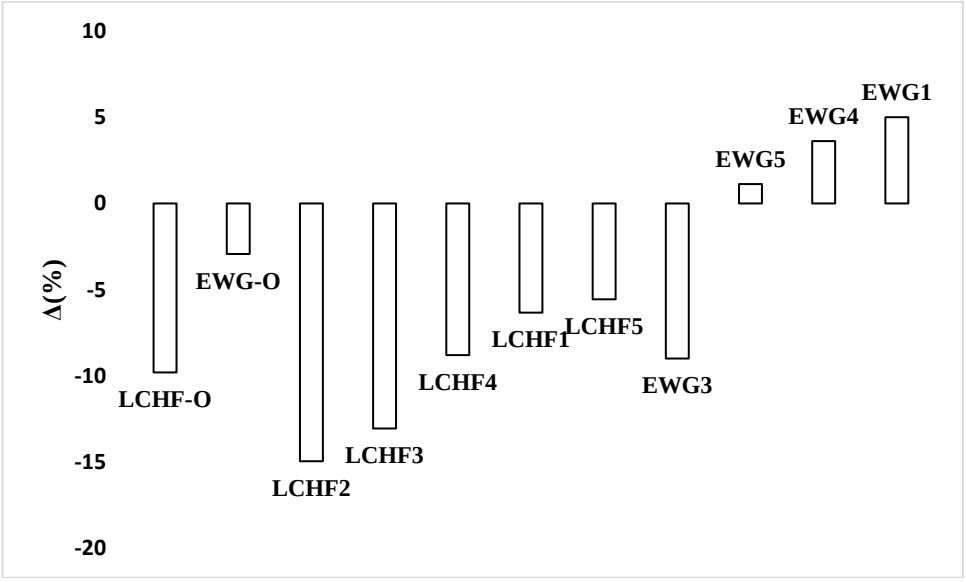


Figure 3.24 – Individual percentage changes in diastolic blood pressure from baseline to endpoint
EWG-O – Eatwell Guide overall group; LCHF-O – low-carbohydrate, high-fat overall group
Data for participant EWG2 not shown due to extremely low DBP measurement at baseline.

### 3.6.7 Levels of carbohydrate and saturated fat intake and individual responses for surrogate markers of cardiometabolic risk

#### 3.6.7.1 Intake of saturated fatty acids and surrogate markers of cardiometabolic risk in the LCHF group

Some individuals in the LCHF group presented with their lowest concentrations/values of significant risk markers when their SFA was the highest. These responses were most pronounced for LDL-C. For 60% of individuals consuming large amounts of SFA resulted in their lowest LDL-C levels, for 40% such high SFA consumption had the opposite effect. High SFA intake resulted in their lowest sdLDL-C concentrations for 80% of individuals. Both insulin and HOMA2-IR were at their lowest point for 60% of individuals when they ate the most SFA (Table 3.25).

Table 3.25 – Levels (peak or minimum) of cardiometabolic risk markers when total saturated fatty acid intake at maximum (significant markers only)

| **Total SFA** | **LCHF1** (16%TE) | **LCHF2** (25%TE) | **LCHF3** (35%TE) | **LCHF4** (27%TE) | **LCHF5** (22%TE) |
|---|---|---|---|---|---|
| **LDL-C** | ↓ | ↓ | ↓ | ↑ | ↑ |
| **sdLDL-C** | ↓ | ↓ | ↓ | ↓ | - |
| **TG** | - | - | ↓ | - | - |
| **INS** | ↓ | ↓ | ↓ | - | - |
| **HOMA2-IR** | ↓ | ↓ | ↓ | - | - |
| **LEP** | ↓ | ↓ | - | - | - |
| **ADPN** | - | ↓ | ↑ | - | - |
| **FGF21** | - | - | - | - | - |
| **SBP** | - | - | ↓ | - | ↓ |
| **DBP** | ↓ | - | - | - | - |

ADPN – adiponectin; DBP – diastolic blood pressure; FGF21 – Fibroblast growth factor 21; HOMA2-IR – Homeostatic model assessment insulin resistance; INS – insulin; LCHF – low-carbohydrate, low-fat; LDL-C – low-density lipoprotein cholesterol; LEP – leptin; SBP – systolic blood pressure; sdLDL – small dense low-density lipoprotein cholesterol; SFA – saturated fatty acids; TG – triglycerides; ↓ - lowest concentration/value for individual during intervention; ↑ - highest concentration/value for individual during intervention

The contribution of selected food groups consumed by individual participants in the LCHF group to maximum total SFA intake differed. Nonetheless, in the majority of participants dairy products made the biggest contribution followed by meat and meat products, whereas nuts and seeds, fish and seafood, butter and other food groups made smaller contributions (Figure 3.25).

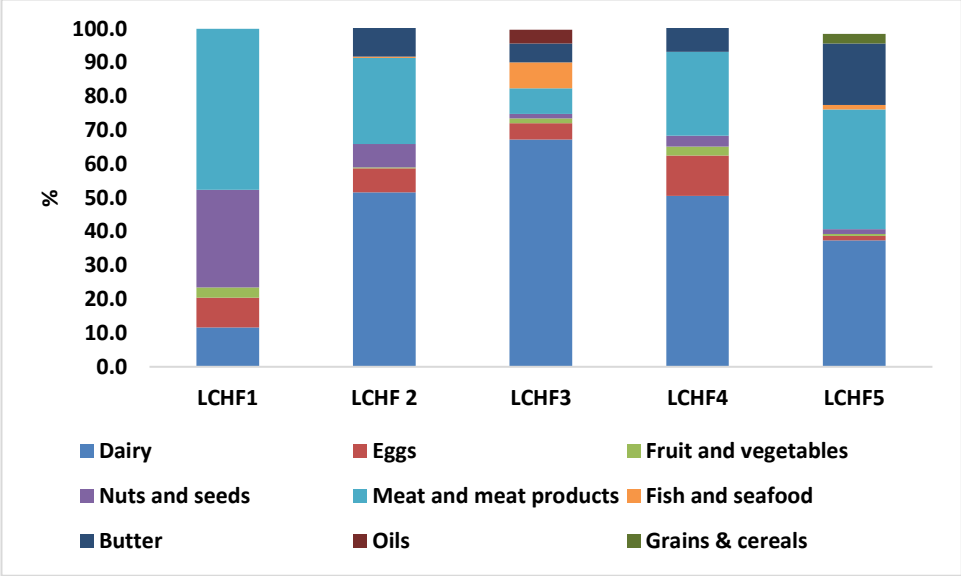


Figure 3.25 – Intake of individual food groups contributing to total maximum saturated fatty acids intake

In all participants in the LCHF arm palmitic acid (C16:0) followed by stearic acid (C18:0) and myristic acid (C14:0) were the biggest even-chained contributors to total saturated fatty acid intake. In 80% of participants contribution was distributed very similarly across even-chained SFAs (Figure 3.26).

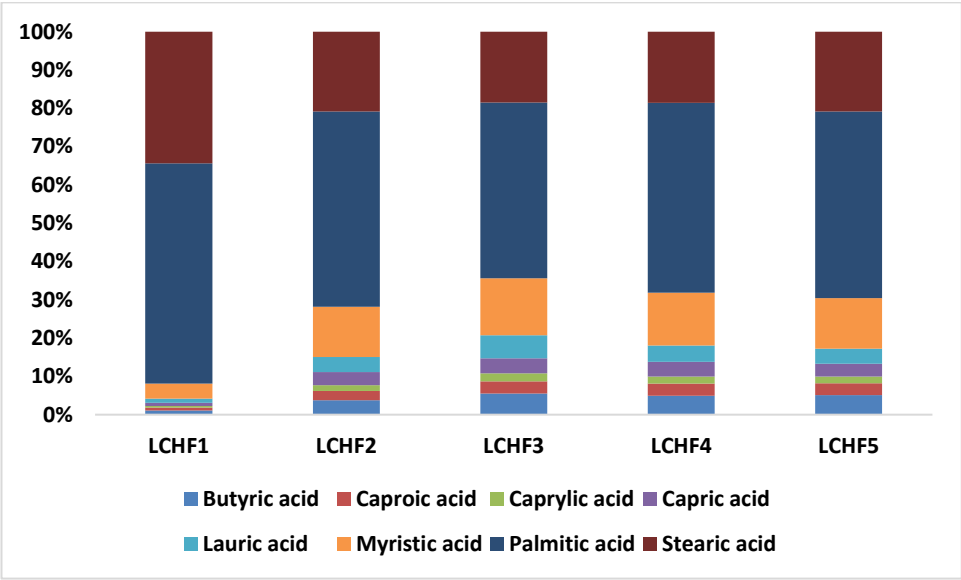


Figure 3.26 – Intake of individual even-chained saturated fatty acids when total saturated fatty acid intake at maximum level

The two individuals where maximum SFA intake resulted in maximum LDL-C concentrations responded the opposite way when their SFA intake was at its lowest. In 80% of individuals minimum SFA consumption led to the highest concentrations of sdLDL-C. Both insulin and HOMA2-IR were at their highest when SFA was minimal for 80% of individuals in the LCHF group. Eighty percent of individuals presented with their lowest

leptin concentrations and 80% presented with their highest FGF21 concentrations at this time point. Lowest intake of SFA also led to the highest SBP and DBP values in 80% of individuals, although only in 60% measurements were at their highest at the same time (Table 3.26).

Table 3.26 – Levels (peak or minimum) of cardiometabolic risk markers when total saturated fatty acid intake at minimum (significant markers only)

| **Total SFA** | **LCHF1** (10%TE) | **LCHF2** (12%TE) | **LCHF3** (12%TE) | **LCHF4** (10%TE) | **LCHF5** (14%TE) |
|---|---|---|---|---|---|
| **LDL-C** | - | - | - | ↓ | ↓ |
| **sdLDL-C** | ↑ | - | ↑ | ↑ | ↑ |
| **TG** | - | ↓ | ↑ | ↑ | - |
| **INS** | - | ↑ | ↑ | ↑ | ↑ |
| **HOMA2-IR** | - | ↑ | ↑ | ↑ | ↑ |
| **LEP** | ↑ | ↑ | - | ↑ | ↑ |
| **ADPN** | ↑ | - | ↓ | - | - |
| **FGF21** | ↑ | ↑ | ↑ | ↑ | ↓ |
| **SBP** | ↑ | ↑ | ↑ | - | ↑ |
| **DBP** | - | ↑ | ↑ | ↑ | ↑ |

ADPN – adiponectin; DBP – diastolic blood pressure; FGF21 – Fibroblast growth factor 21; HOMA2-IR – Homeostatic model assessment insulin resistance; INS – insulin; LCHF – low-carbohydrate, low-fat; LDL-C – low-density lipoprotein cholesterol; LEP – leptin; SBP – systolic blood pressure; sdLDL – small dense low-density lipoprotein cholesterol; SFA – saturated fatty acids; TG – triglycerides; ↓ - lowest concentration/value for individual during intervention; ↑ - highest concentration/value for individual during intervention

In 80% of participants in the LCHF arm, when SFA intake was at its minimum over the course of the intervention (which was at baseline for all of them), meat and meat products were the largest contributors to total SFA intake. Dairy and dairy products were the second biggest contributors. Foods attributed to the grains and cereals category were also contributors to SFA intake and those contributing higher amounts of SFA tended to be food items such as cakes and biscuits (Figure 3.27).

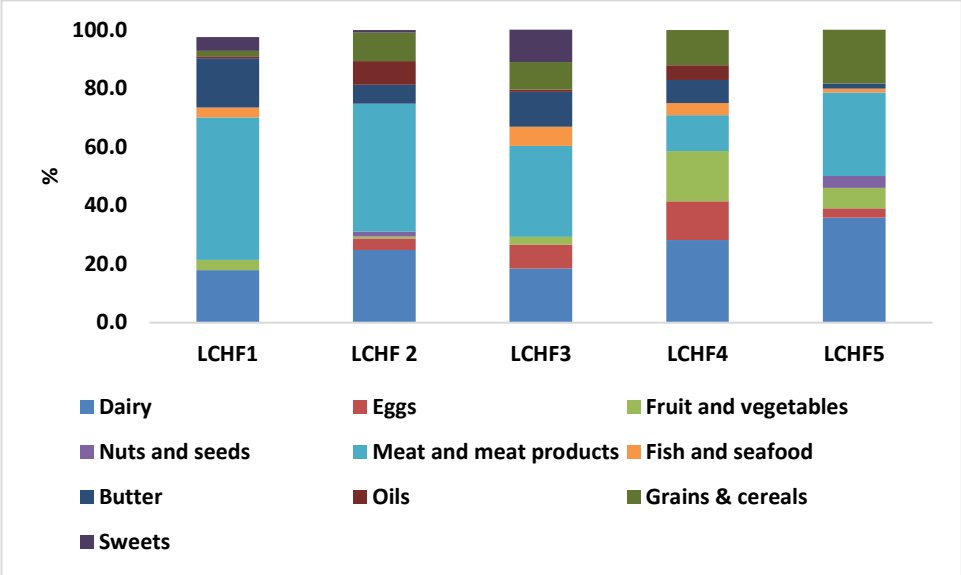


Figure 3.27 – Intake of individual food groups contributing to total minimum saturated fatty acids intake

In the majority of participants minimum saturated fatty acid intake came from firstly palmitic acid, secondly stearic acid and thirdly myristic acid. In 80% of participants palmitic acid intake was nearly double that of stearic and myristic acid intake combined (Figure 3.28).

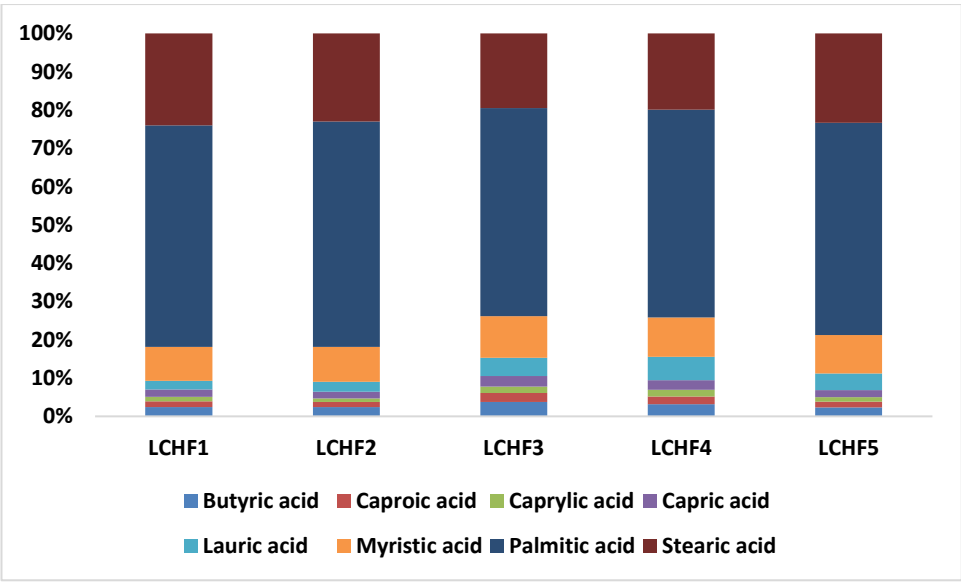


Figure 3.28 – Intake of individual even-chained saturated fatty acids when total saturated fatty acid intake at minimum level

### 3.6.7.2 Intake of carbohydrates and surrogate markers of cardiometabolic risk in the EWG group

Maximum CHO intake resulted in 80% of individuals in the EWG group in their lowest LDL-C concentrations. Sixty percent of individuals presented with their lowest TG concentrations. Twenty percent had their highest insulin and HOMA2-IR values but 60% their lowest. Maximum CHO consumption resulted in the highest FGF21 levels in 40% of individuals. Forty percent presented with their highest SBP and DBP and 40% presented with their lowest SBP and DBP. Maximum CHO consumption meant for 40% of individuals that their leptin concentrations reached a maximum, but for another 40% the opposite was the case (Table 3.27).

Table 3.27 – Levels (peak or minimum) of cardiometabolic risk markers when total carbohydrate intake at maximum (significant markers only)

| Total CHO | EWG1 (51%TE) | EWG2 (48%TE) | EWG3 (50%TE) | EWG4 (34%TE) | EWG5 (58%TE) |
|---|---|---|---|---|---|
| **LDL-C** | ↓ | ↓ | ↓ | ↑ | ↓ |
| **sdLDL-C** | - | - | - | - | - |
| **TG** | - | - | ↓ | ↓ | ↑ |
| **INS** | ↓ | ↓ | ↓ | - | ↑ |
| **HOMA2-IR** | ↓ | ↓ | ↓ | - | ↑ |
| **LEP** | ↓ | ↓ | ↑ | - | ↑ |
| **ADPN** | - | ↓ | - | - | - |
| **FGF21** | ↑ | - | ↓ | ↑ | - |
| **SBP** | ↑ | ↓ | ↓ | ↑ | ↓ |
| **DBP** | ↑ | ↓ | - | ↑ | ↓ |

ADPN – adiponectin; CHO – carbohydrates; DBP – diastolic blood pressure; FGF21 – Fibroblast growth factor 21; HOMA2-IR – Homeostatic model assessment insulin resistance; INS – insulin; LCHF – low-carbohydrate, low-fat; LDL-C – low-density lipoprotein cholesterol; LEP – leptin; SBP – systolic blood pressure; sdLDL – small dense low-density lipoprotein cholesterol; TG – triglycerides; ↓ - lowest concentration/value for individual during intervention; ↑ - highest concentration/value for individual during intervention

In 80% of participants in the EWG arm white grains and cereals made the biggest contribution to total CHO intake, followed by fruit and vegetables. Dairy and wholegrain products were also contributors but far less so than the main two contributing food groups. Some individuals also reported sweets and snacks consumption and intake of non-alcoholic beverages, which contributed to total CHO intake, mainly through added sugars (e.g. in tea, coffee of sugar-sweetened beverages (SSBs) (Figure 3.29).

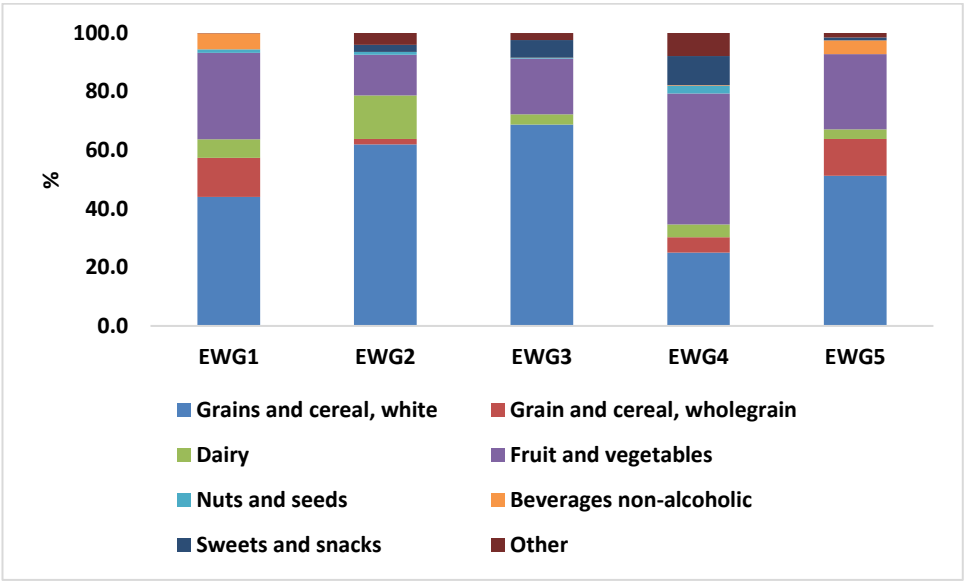


Figure 3.29 – Intake of individual food groups contributing to total maximum carbohydrate intake

In the majority of participants in the EWG arm starches contributed the most to maximum CHO intake, followed by total sugars (Figure 3.30).

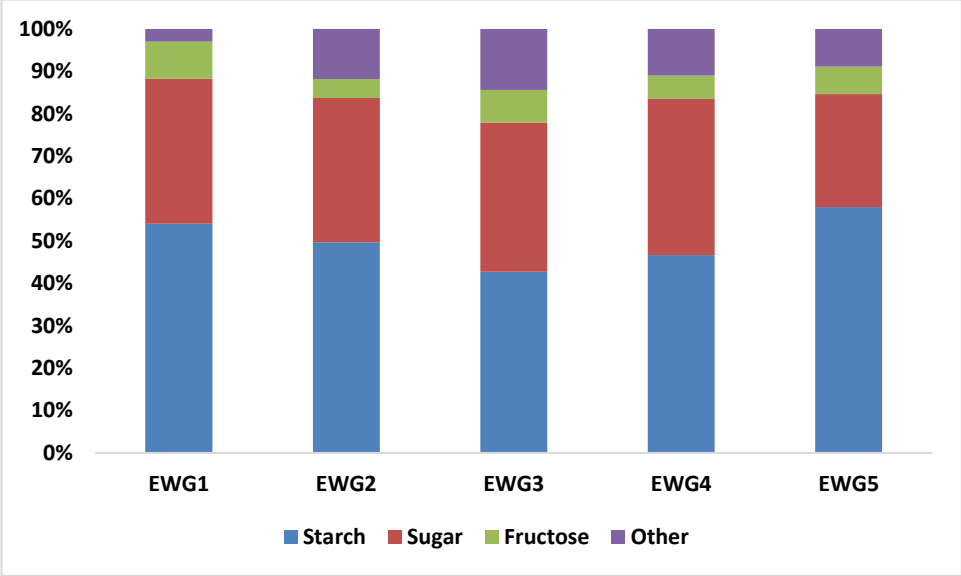


Figure 3.30 – Intake of selected carbohydrates when total carbohydrate intake at maximum level

When total CHO intake was at its minimum sdLDL-C concentrations and SBP measurements reached their peak in 60% of EWG individuals. Forty percent presented with their highest insulin concentrations and HOMA2-ir values at this point whilst for 20% of participants the opposite occurred. Leptin concentrations reached their peak with minimum CHO intake in 40% of individuals, whilst adiponectin concentrations were at their minimum in 60% of individuals. Minimum intake of CHO resulted in lowest FGF21 concentrations in 40% of individuals (Table 3.28).

Table 3.28 – Levels (peak or minimum) of cardiometabolic risk markers when total carbohydrate intake at minimum (significant markers only)

| **Total CHO** | **EWG1** (36%TE) | **EWG2** (35%TE) | **EWG3** (41%TE) | **EWG4** (33%TE) | **EWG5** (51%TE) |
|---|---|---|---|---|---|
| **LDL-C** | - | - | - | - | - |
| **sdLDL-C** | ↑ | ↑ | - | - | ↑ |
| **TG** | ↑ | ↓ | - | - | - |
| **INS** | - | ↑ | ↑ | ↓ | - |
| **HOMA2-IR** | - | ↑ | ↑ | ↓ | - |
| **LEP** | ↑ | ↑ | - | - | - |
| **ADPN** | ↓ | ↑ | ↓ | ↓ | - |
| **FGF21** | - | ↓ | - | ↓ | ↑ |
| **SBP** | - | ↑ | ↑ | - | ↑ |
| **DBP** | - | - | ↑ | - | ↑ |

ADPN – adiponectin; CHO – carbohydrates; DBP – diastolic blood pressure; FGF21 – Fibroblast growth factor 21; HOMA2-IR – Homeostatic model assessment insulin resistance; INS – insulin; LCHF – low-carbohydrate, low-fat; LDL-C – low-density lipoprotein cholesterol; LEP – leptin; SBP – systolic blood pressure; sdLDL – small dense low-density lipoprotein cholesterol; TG – triglycerides; ↓ - lowest concentration/value for individual during intervention; ↑ - highest concentration/value for individual during intervention

In 60% of participants in the EWG arm white grains and cereals contributed the most to total CHO intake followed by fruit and vegetables when these individuals consumed the least CHO. In 20% of participants fruit and vegetables made the highest contribution and in 20% wholegrain products contributed the most (followed by fruit and vegetables) (Figure 3.31).

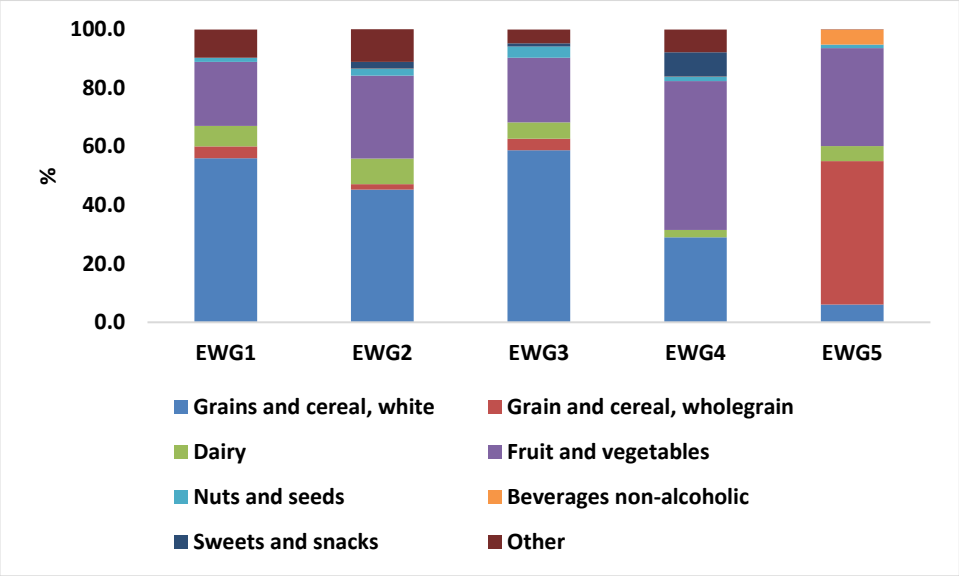


Figure 3.31 – Intake of individual food groups contributing to total minimum carbohydrates intake

In 60% of participants in the EWG arm starch intake was lower at minimum total CHO intake than at maximum total CHO intake. In 40% of participants total sugars were the main contributor to total CHO intake. Twenty percent of participants do not seem to have consumed any dairy products as only fructose but no other sugars contributed to total CHO intake pointing at no lactose intake, contained in dairy products (Figure 3.32).

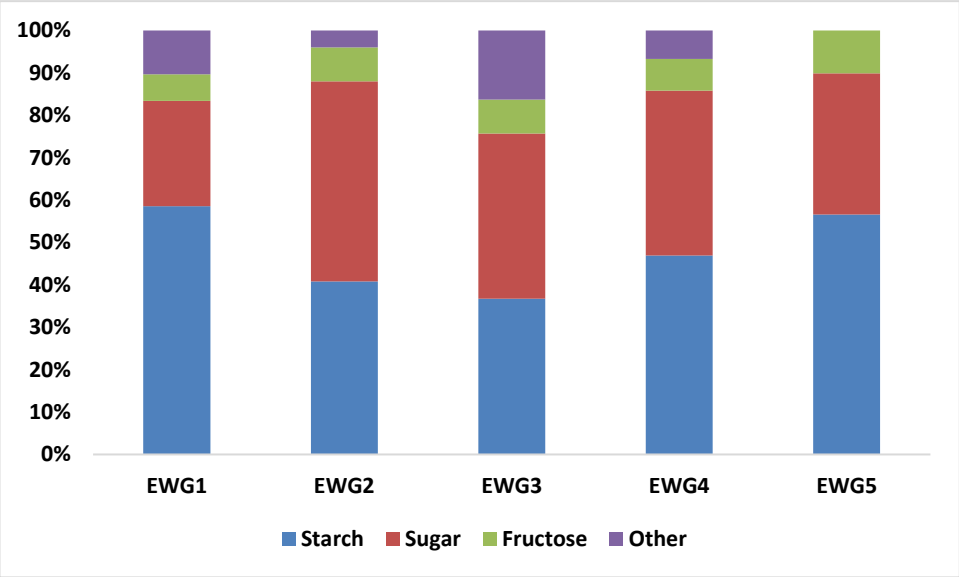


Figure 3.32 – Intake of selected carbohydrates when total carbohydrate intake at minimum level

### 3.6.8 Body composition

#### 3.6.8.1 Overall changes to adiposity proxies

There were significant changes for all adiposity proxies presented in this chapter. For WC and VAT there were significant effects of diet ($p$=.019 and $p$=.032 respectively), time (both $p$<.0001) and diet-time interaction ($p$=.029 and $p$=.031 respectively) (Figure 3.33 and Figure 3.34). For FM (%) there were significant effects of time ($p$=.005) and diet-time interaction ($p$=.003) (Figure 3.35). For NC only the effect of time was significant ($p$=.001) (Figure 3.33). Throughout the study the LCHF group presented with significantly higher FM (%), WC, NC and VAT than the EWG group and measurements remained greater in the LCHF group at endpoint. However, the LCHF presented with significantly greater reductions in VAT and WC than the EWG group. The EWG had slight increases in FM (%). Mean FM decreased continuously in the LCHF group between baseline and endpoint but slightly increased in the EWG group after an initial decrease. Mean VAT volume showed a steady decline in both diet groups between baseline and endpoint, but more so in the LCHF than in the EWG group.

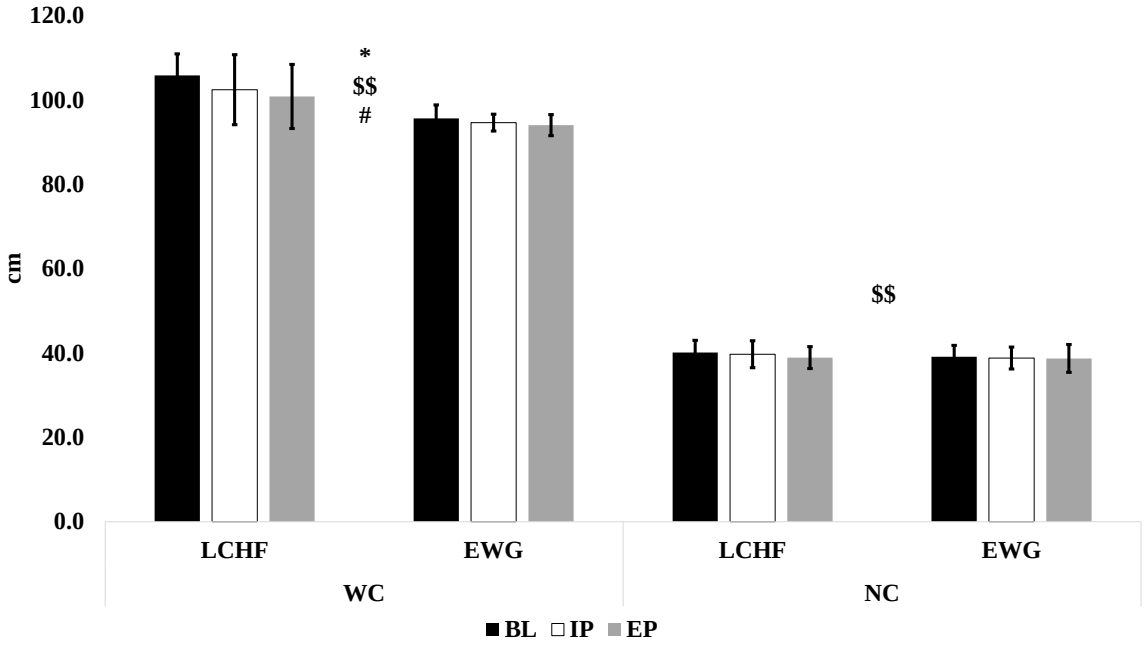


Figure 3.33 –Waist circumference and neck circumference at baseline, interim point and endpoint (Mean±SD)
EWG- Eatwell Guide; LCHF – low-carbohydrate, high-fat; NC – neck circumference; WC – waist circumference; *p<.05 for diet; $$p<.005 for time; #p<.05 for diet*time; all data based on n=5 in LCHF group and n=5 in EWG group

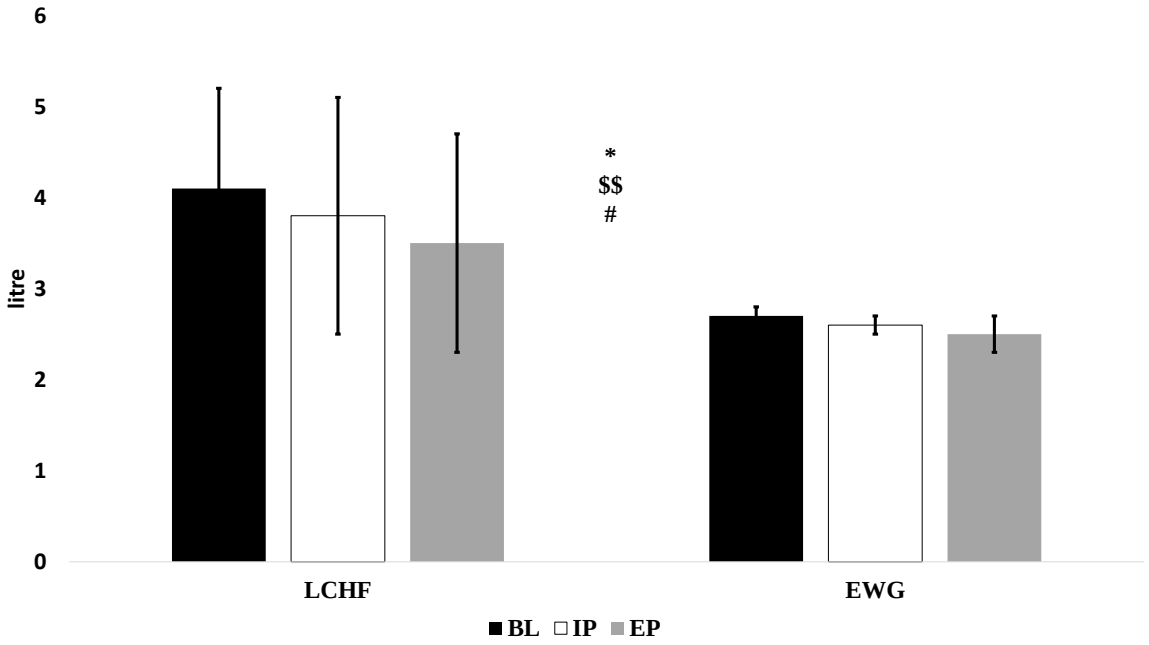


Figure 3.34 –Visceral adipose tissue volume at baseline, interim point and endpoint (Mean±SD);
EWG- Eatwell Guide; LCHF – low-carbohydrate, high-fat; *p<.05 for diet; $$p<.005 for time; #p<.05 for diet*time; all data based on n=5 in LCHF group and n=5 in EWG group

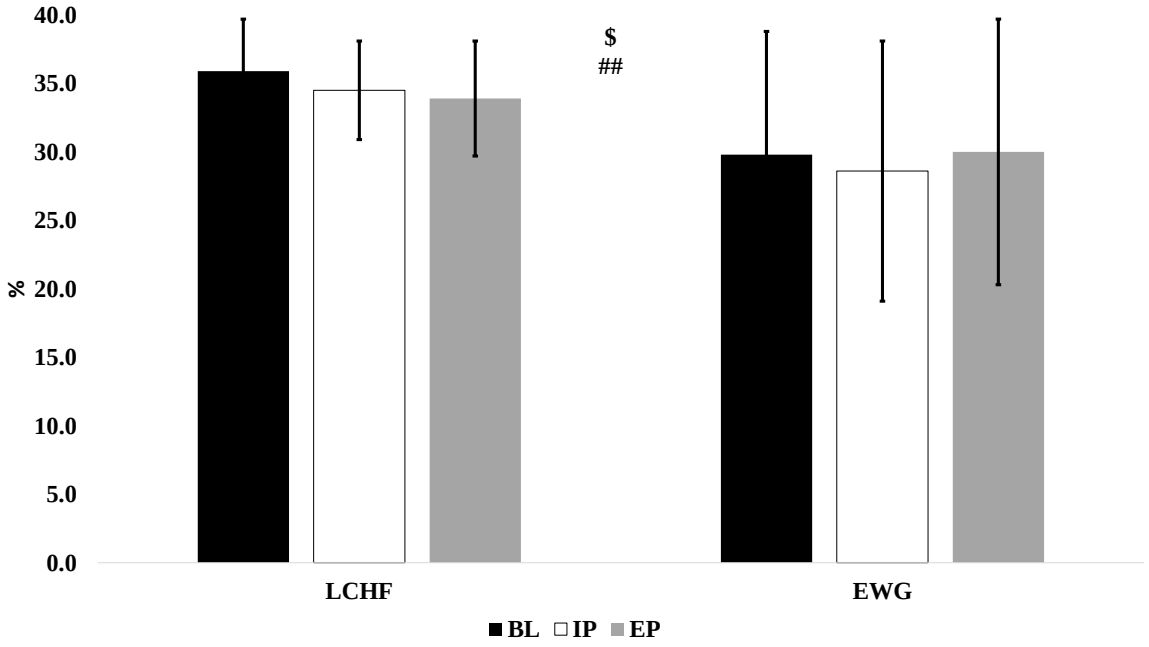


Figure 3.35 –Percent fat mass at baseline, interim point and endpoint (Mean±SD);
EWG- Eatwell Guide; LCHF – low-carbohydrate, high-fat; $p<.05 for time; ##p<.05 for diet*time; all data based on n=5 in LCHF group and n=5 in EWG group

### 3.6.8.2 Individual responses in changes in adiposity proxies

All participants in the LCHF group decreased their WC by between 3% and 9%. The majority of EWG participants decreased their WC by between 1% and 4%. One participant presented with a slight increase in WC by 0.3% (Figure 3.36).

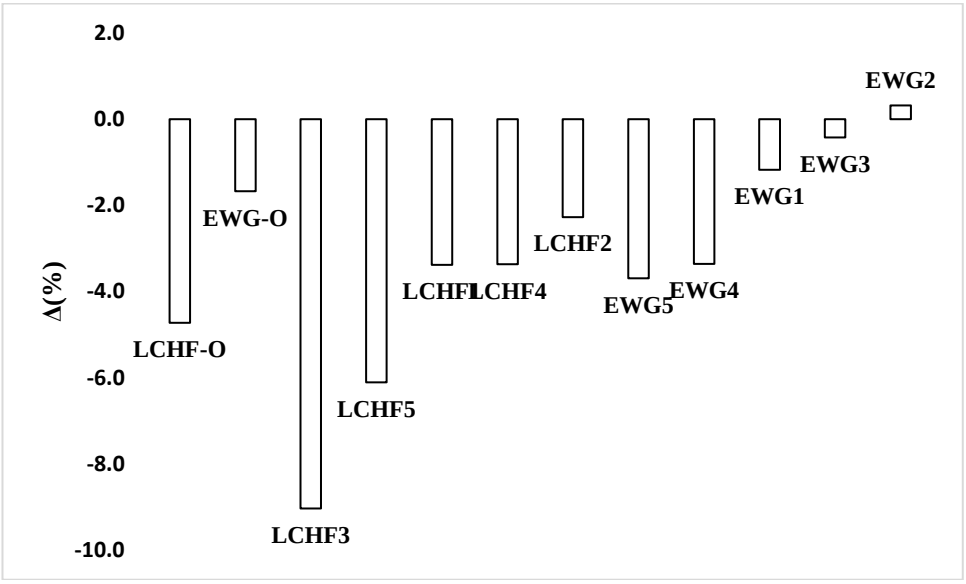


Figure 3.36 – Individual percentage changes to waist circumference from baseline to endpoint
EWG-O – Eatwell Guide overall group; LCHF-O – low-carbohydrate, high fat overall group; all data based on n=5 in LCHF group and n=5 in EWG group

With regards to NC all participants in the LCHF group presented with reductions of between 2% and 4%, whilst the majority in the EWG group reduced their NC by ≤3%. One participant in the EWG group presented with a by 1% increased NC (Figure 3.37).

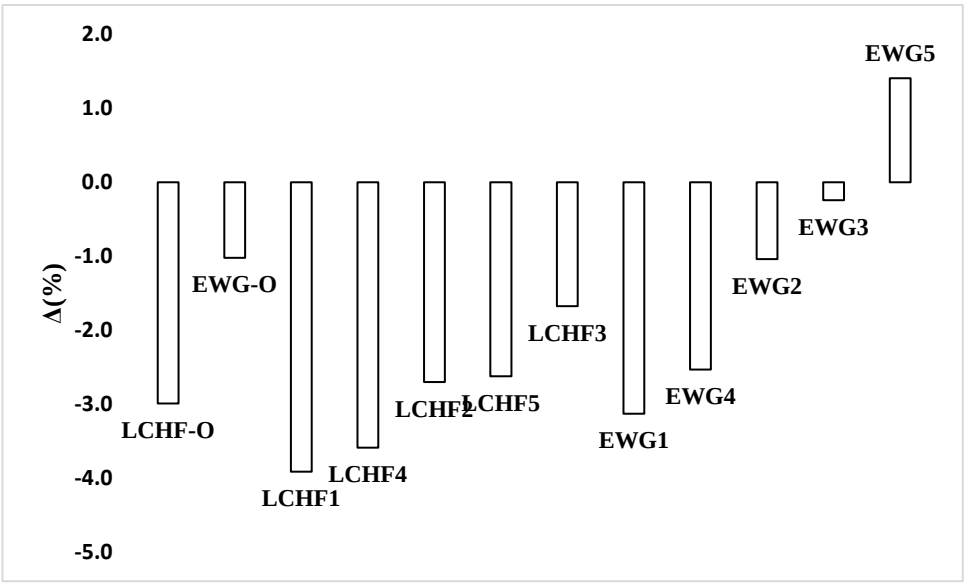


Figure 3.37 – Individual percentage changes to neck circumference from baseline to endpoint
EWG-O – Eatwell Guide overall group; LCHF-O – low-carbohydrate, high fat overall group; all data based on n=5 in LCHF group and n=5 in EWG group

One participant in the EWG group presented with an unchanged volume of VAT, whereas all the other participants had significant decreases between 7% and 15%. The decreases were significantly greater in the LCHF group, which presented with reductions of between 8%

and 32%. For two LCHF participants the decreases were smaller compared to those in the EWG group (Figure 3.38).

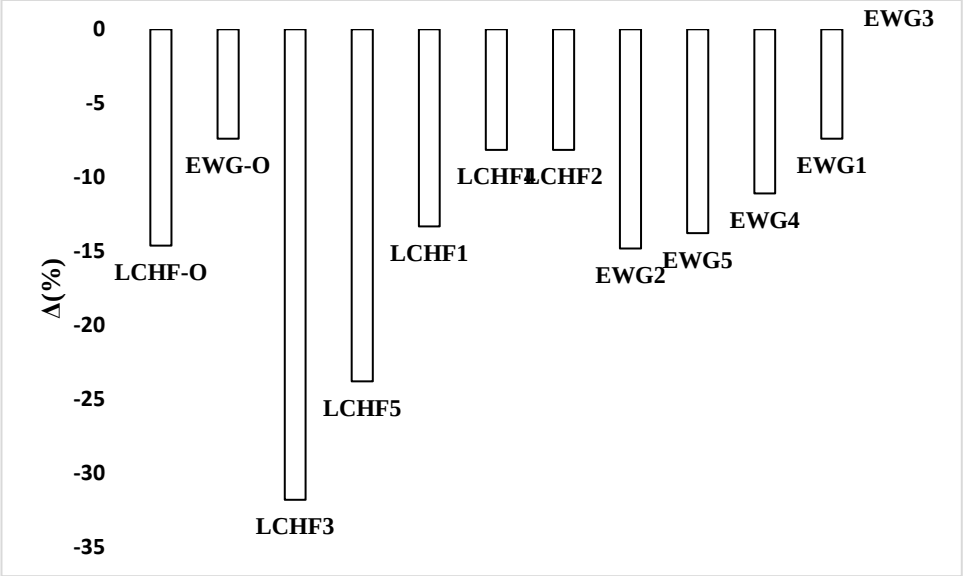


Figure 3.38 – Individual percentage changes to visceral adipose tissue volume from baseline to endpoint
EWG-O – Eatwell Guide overall group; LCHF-O – low-carbohydrate, high fat overall group; Δ(%)
EWG3=0; all data based on n=5 in LCHF group and n=5 in EWG group

All participants in the LCHF group had significantly and greater reductions in FM than the EWG group. Reductions were between 3% and 12%. In fact, only one participant in the EWG group presented with decreases in fat mass (at 3% albeit smaller than any of those in the LCHF group), the other EWG participants had increases of ≤3% (Figure 3.39).

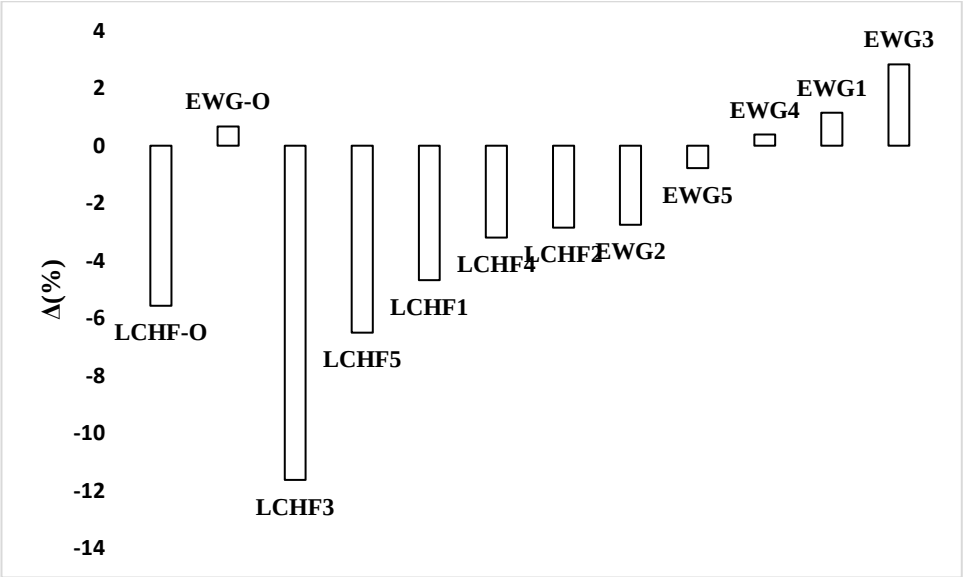


Figure 3.39 – Individual percentage changes to fat mass from baseline to endpoint
EWG-O – Eatwell Guide overall group; LCHF-O – low-carbohydrate, high fat overall group; all data based on n=5 in LCHF group and n=5 in EWG group

### 3.6.9 Physical activity

#### 3.6.9.1 Pre-study moderate-to-vigorous physical activity levels

There were no significant differences between groups ($p$=.913). On average, the EWG group reported to spend more time in MVPA than the LCHF group before they started the intervention but the standard deviation was very high (Table 3.29).

Table 3.29 – Self-reported pre-study mean daily total moderate-to-vigorous physical activity (M ±SD) in LCHF and EWG group

| | LCHF | EWG | P** |
|---|---|---|---|
| **Total MVPA (hours/day)*** | 2.2 ±0.8 | 2.7 ±2.4 | .913 |

EWG – Eatwell Guide; LCHF = Low-carbohydrate, high-fat; MVPA = Moderate-to-vigorous physical activity, *adjusted for baseline BMI, **p values associated with type 3 tests of fixed effects

#### 3.6.9.2 Intervention moderate-to-vigorous physical activity levels

There were no significant effect for diet, time or diet*time interaction for the daily amount spent at MVPA (all $p \geq .05$) (Table 3.30). Over the course of the intervention the LCHF group increased their activity levels by 34% but the standard deviation more than doubled. After an initial increase in MVPA time in the EWG group, a decrease occurred between interim point and endpoint so that overall the EWG group increase their MVPA time by 2%. Standard deviation in the EWG group also increased.

Table 3.30 – Moderate-to-vigorous physical activity levels at baseline, interim point and endpoint

| | MVPA (min/d)* | | | | |
|---|---|---|---|---|---|
| | **BL** | **IP** | **EP** | **p**** | **F***** |
| **LCHF** | 213.6 ±45.7 | 226.3 ±52.1 | 286.4 ±118.3 | Diet: .392 | 1.10.043 |
| **EWG** | 202.0 ±52.6 | 220.1 ±76.7 | 205.3 ±79.4 | Time: .191<br>Diet*Time: .198 | 2,12.278<br>2,12.278 |

BL – Baseline, EP – endpoint, EWG – Eatwell Guide, IP – interim point, LCHF – low-carbohydrate, high-fat, min/d – minutes per day, MVPA – Moderate-to-vigorous physical activity, *adjusted for daily wear time and BMI at baselines, interim point and endpoint respectively, **p values associated with type 3 tests of fixed effects, *** F(1,10.043) for diet, F(2,12.278) for time, F(2,12.278) for diet*time

### 3.6.9.3 Differences between pre-study and intervention physical activity levels

There were no significant differences between pre-study and baseline MVPA time reported by the participants in the EWG group ($p$=.424), although self-reported MVPA was lower than objectively measured MVPA. Accelerometry-assessed MVPA time in the LCHF group was also higher than self-reported MVPA time and this difference was statistically significant ($p$=.024) (Table 3.31).

Table 3.31 – Comparison between pre-study and baseline time spent at moderate-to-vigorous physical activity (M ±SD)

| | MVPA (hours/day) | | |
|---|---|---|---|
| | **PRE** | **BL*** | **p** |
| **LCHF** | 2.2 ±0.8 | 3.6 ±0.8 | .024 |
| **EWG** | 2.7 ±2.4 | 3.4 ±0.9 | .424 |

BL – Baseline (ActiGraph GT9X); EWG – Eatwell Guide; LCHF – Low-carbohydrate, high-fat; MVPA – Moderate-to-vigorous-physical-activity, PRE – pre-study (RPAQ)
*converted from MVPA (min/day) as MVPA(min/day)/60

## 3.7 Discussion

### 3.7.1 Dietary intake, adherence and food cravings

Although allocation to the two different diets was randomised in some respects the pre-study dietary habits of those allocated to the LCHF group seemed to pre-empt that it might be relatively easy to embrace some of the rules of the LCHF diet. This was because of the significantly higher intakes of meat and meat products and non-significantly higher intakes of vegetables in this group. However, at the same time they reported higher intakes of cereals & cereal products and potatoes, which are not permitted or at least highly restricted as part of a LC lifestyle (Volek et al., 2009a). Likewise, whilst the higher consumption of fruit in the EWG group seemed make a positive contribution to the CHO intake in this group, this was still below EWG recommendations and had to be increased, which meant that some protein and total fats would need to be exchanged for CHO-rich foods. The increase in total fat intake (%TE) in the LCHF group seemed to have been accompanied by the selection of high-quality fats. Whilst the intake of SFA (%TE) nearly doubled between baseline and endpoint, the majority of fats came from unsaturated sources. Similar findings were made in previous studies, in which participants that could eat ad libitum consumed more USFA than SFA (Sharman et al., 2004; Volek et al., 2003)

According to their FDs the LCHF group overall seemed to have adhered to restricting their intake of CHO to between 30g and 50g per day, although this was better achieved at interim point than at endpoint as demonstrated by the SD, which was 14.9 g/d and 22.1 g/d respectively. The findings that none of the LCHF participants had ketone concentrations high enough to be in nutritional ketosis at endpoint however seems to point at higher levels of CHO consumed. Nonetheless, it can be assumed that all participants at interim point and endpoint met with the requirements of a low-carbohydrate diet and consumed <130 g/d of CHO, unless the extent of underreporting was extreme.

A recent systematic review and meta-analysis of interventions with a duration of between three months and 48 weeks conducted by Huntriss et al. (2018) on the use of LC diets for the management of T2DM in adults <18 years (n=1957) concluded that CHO intake of <50 g/d seems to be hard to achieve at least in this particular population. This was indicated by poor dietary adherence, whereas reducing CHO intake to <130 g/d seems to be more realistic. In none of the 11 trials included in the review prescribing either a LC or a ketogenic diet participants managed to adhere to the CHO prescription by endpoint with participants in one trial remaining <50 g/d but not achieving the prescribed <20 g/d over 24 weeks. In nine trials participants managed to consume <130 g/d. No demographic baseline participant

characteristics were reported. These findings mirror the ones from the present study, although the sample in the present study had not been diagnosed with CMD.

Hallberg et al. (2018a; 2018b) conducted a one-year trial prescribing a personalised LC diet (CHO ~30 g/d to induce nutritional ketosis as measured daily via blood hydroxybutyrate levels) to 186 overweight or obese patients with T2DM (study completers). They found that at the 70 day mark nutritional ketosis was on average achieved, and whilst this was no longer the case after one year, hydroxybutyrate levels were still nearly twice as high than at baseline (0.3 mmol/L). Participants in this intervention were heavily supported through an online app and tele access to a health coach and a medical professional. They also received personalised nutritional coaching and had access to an online support community. The research team noted that the regular monitoring of hydroxybutyrate levels might have acted as positive re-enforcement.

In the recently conducted 12-months DIETFITS RCT (Gardner et al., 2018; Stanton et al., 2017) 304 overweight or obese adults (age 18 – 50 y) were allocated to a LC diet. They were asked to attempt and achieve consumption of as little as 20 g/d of CHO as quickly as possible within the first eight study weeks and maintain these levels for a few more weeks after this. However, this was not compulsory to remain part of the trial. During the initial eight-week period participants could utilise a number of online and offline methods to record dietary intake and self-monitor achievement of their goal. After the first eight weeks participants were instructed to gradually increase CHO intake with the goal to keep this as low as possible whilst retaining palatability, satiety and enjoyment. Twenty-two group evening classes over the course of the 12 months provided dietary information and worked with a behavioural modification strategy. Additionally, a number of psychosocial questionnaires on general health and eating behaviour had to be completed. After the first four study cohorts reported that adherence was getting harder after six month, the final cohort received regular text messages after the six-months mark. Three 24h recalls (2 weekday, 1 weekend day) were collected at four study time points. It was not reported how many of the participants managed to achieve a CHO intake <20 g/d during the first eight-week period. At six months mean daily CHO intake was <130 g/d (26.5%TE) and at 12 months just above this threshold (132.4g/d or 29.8%TE), providing evidence that this group were more comfortable with CHO amounts above those that induce nutritional ketosis but well below official dietary recommendations.

A study conducted by Saslow et al. (2014 and 2017) asked 16 pre-diabetic and diabetic overweight adults to follow a ketogenic diet for 12 months. Nutritional ketosis was self-tested twice a week via blood ketone test strips to determine hydroxybutyrate levels for the

first few months of the study but not thereafter. Participants were instructed to lower their CHO intake until they had achieved ketone levels >0.5 mmol/L. By week four 55% and by week six 75% of participants reported to be in nutritional ketosis. Participants took part in regular nutrition and health behaviour education classes (12 within the first three months and an additional 7 classes over the next nine-months period) that also addressed physical activity, sleep and socio-psychological topics. Food intake was assessed at four time points via 24h recall. At three months 57% of participants consumed CHO <50 g/d with mean consumption for the group at 57.8 g/d (SD 41.5), at six month mean intake of CHO was also <50 g/d (95%CI 27.4, 60.8) but at 12 months this had increased to 73.7 g/d (95% CI 51.5, 96.0). Similar to other studies and indeed the present study dietary adherence to achieve levels maintaining nutritional ketosis seems to be difficult to obtain for participants.

A few studies have explored physiological and behavioural aspects of adherence to ketogenic or low-carbohydrate diets. McClain et al. (2013) conducted a secondary analysis of data collected from 42 overweight or obese women in a previous trial to investigate whether dietary adherence in the Atkins Diet depended on IR status of participants at baseline but found no significant results. Yancy et al. (2015) found that obese participants with or without T2DM (n = 61) when given a choice to follow a LC diet based on food preferences had similar adherence levels at 12 weeks (92.8%) and at 24 weeks (92.9%). At 48 weeks, 90.6% of participants still adhered to the diet. However, overall these results were not significantly different from other groups that were randomised to a particular diet. Yancy et al. (2015) therefore suggested that other factors but dietary preference should determine future diet allocation. A recent systematic review and meta-analysis by Leavy et al. (2018) also concluded that choice of diet did not significantly impact adherence. McVay et al. (2015) assessed impact of pre-study level of macronutrient intake for 71 obese adults with or without T2DM on dietary adherence to a LC diet (ketogenic induction of <20 g/d of CHO for the first couple of weeks rising by 5 g/d whilst approach their target weight or when experiencing food cravings). Adherence was assessed via self-reported food records. Only 26% percent adhered to the prescription by the end of week two, whilst another 28% consumed 20-30 g/d of CHO. Mean CHO intake at two weeks was 40.1 g/d. By week 48 daily mean CHO consumption steadily increased to 62.0 g/d. Pre-study macronutrient levels did not significantly affect dietary adherence, similar to the findings in the present study.

The EWG group did not manage to change the composition of their diet sufficiently to reach a total mean intake of CHO of 50%TE for the group. Consequently, mean total CHO %TE intake for the group as a whole fell into the moderate category. Overall intake of total fats

and SFA (%TE) remained consistent throughout the intervention and within EWG recommendations.

That adherence was also an issue in the EWG group is in line with previous research. Studies reporting on adherence to dietary guidelines tend to be cross-sectional and observational, rather than interventional. The NDNS showed that in none of the survey years 2008 - 2016 mean CHO intake of UK adults (aged 19-64 years) met the UK recommendations (Bates et al., 2014; Bates et al., 2016; Roberts et al., 2018). Several other European cross-sectional and longitudinal studies in Luxembourg, Spain and Switzerland have also reported lack of adherence to national dietary guidelines whether these were nutrient-based or food-based (Alkerwi et al., 2012; De Abreu et al., 2013; Rodriguez-Rodriguez et al., 2017; Schneid Schuh et al., 2018; Schneid Schuh et al., 2019). A systematic review and meta-analysis by Haack and Byker (2014) including 25 mainly observational studies with up to 215,000 participants found that adherence to US dietary guidelines was poor, especially in terms of fruit, vegetable and dairy intake. Sobiecki et al. (2016) analysed data from 30,251 participants from the EPIC study (European Prospective Investigation into Cancer and Nutrition–Oxford Study) to assess adherence to the dietary guidelines using a semi-quantitative FFQ. The sample was divided into four groups categorised as meat eaters, fish eaters, vegetarians and vegan. Only meat eaters fell below the dietary recommendations for CHO whilst the other groups exceeded the recommendations. Both Alkerwi et al. (2012) and Schneid Schuh et al. (2018) found that adherence was poorer in smokers than in non-smokers. However, this limitation did not apply to the participants in the current study as only non-smokers were recruited.

As there seemed to have been issues with dietary adherence in both groups, it was important to examine what might have been a confounding factor in this. Dietary adherence could therefore be closely linked to the experience of food cravings (as recorded through the FCI-UK), which were another secondary outcome in this analysis. It is important to note that cravings are different from hunger as hunger can be satisfied by any energy-dense food, whereas cravings are directed at specific foods. To satisfy cravings foods at least similar to the craved food item need to be consumed (Reichenberger et al., 2018). In controlled feeding studies food cravings were a frequent reason for lack of dietary adherence (Martin et al., 2011). A number of studies have investigated potential associations between CHO and food cravings (Lennerz et al., 2013; Ma et al., 2017; Ventura et al., 2014), and of all food categories in the FCI-UK starchy foods followed by sugary foods were the ones that were craved the most at baseline when CHO intake (%TE) could be classed as moderate in both the LCHF and the EWG group.

There is some evidence that LCHF diets in particular decrease food cravings (Martin et al., 2011). Indeed, in the LCHF group cravings for sugary, fast and starchy foods were at their lowest level at interim point when CHO intake was the lowest in the group. At this point participants also gave in the least to their cravings for sugary and starchy foods. Similar to a diet conducted by Martin et al. (2011), who used the US-version of the FCI, cravings for starchy foods also steeply declined in 134 obese adults who were instructed to follow the New Atkins Diet (with its ketogenic induction phase) over a two-year period. However, a severe limitation to applying Martin et al. (2011) findings to the present study is that dietary intake data were not collected during their research and actual CHO intake by the participants is therefore unknown.

### 3.7.2 Clinical markers of cardiometabolic risk

#### 3.7.2.1 The impact of the intervention on fasting plasma lipids and plasma lipid ratios

Some of the significant findings from the CALIBER study appear to be in accordance with the perceptions of the impact of reduced CHO intake in general and a LCHF diet in particular, others seem to contradict the general consensus. However, it is increasingly recognised that an individual's physiological response to nutrients might be impacted by a number of factors, such as genetic deposition, age, environment, and therefore vary from one person to another (Laddu and Hauser, 2019; Ordovas et al., 2018; Van Ommen et al., 2017). For example, the results for the overall LCHF group for LDL-C seem to add to the body of evidence that a diet low in CHO and high in SFA increases LDL-C concentrations. As LDL-C is a primary target in CV risk reduction and management (Catapano et al. 2016), this would be a cause of concern. Indeed one of the main arguments employed against following a LCHF diet is the perception that this raises LDL-C concentrations and is therefore assumed detrimental to CM health (Brouns, 2018; Mansoor et al., 2016).

A number of studies of short duration of ≤8 weeks prescribing either ketogenic or non-ketogenic LCHF diets did find significant increases in LDL-C levels in healthy normal-weight or overweight men (Urbain et al., 2017; Volek et al., 2000), healthy normal-weight women (Volek et al., 2003) and healthy overweight and obese women (Volek et al., 2004) either under ad libitum or hypocaloric conditions. Contrary to this, Meckling and co-workers (2002) found that LDL-C levels significantly decreased in overweight and obese women over the course of 8 weeks. In a follow-up study conducted by Meckling et al. (2004), 15 overweight and obese adults were asked to follow the same LCHF diet protocol as previously described by Meckling over a 10-week period. This time participants were instructed to also reduce energy intake compared to baseline levels to induce weight loss. Low-density

lipoprotein cholesterol concentrations did not change significantly. In about 50% of participants LDL-C levels initially rose but then dropped, in the majority of cases below baseline levels. Veum and colleagues (2017) made similar findings in their 12-week trial conducted with adult males presenting with abdominal obesity. In the majority of participants LDL-C levels initially increased and then decreased again but these findings were not significant over the course of the study.

Trials of longer duration (≥6 months) also had mixed results with increases occurring in the first few months of the diet to then decrease or level off. These were the findings made by Foster et al. (2010) in a 2-year randomised parallel study in obese adults. LDL-C levels increased over the first three months, they then decreased below baseline levels at 12 months. Although they subsequently increased again over the next year, they remained below baseline levels at 24 months but at this point the results were no longer significant. The authors acknowledged that liberalisation from LCHF dietary restrictions might have occurred over the longer course of the study confounding the findings. Contrary to Foster's results, a one-year RCT of adults with abdominal obesity and at least one more risk factor for MetS found LDL-C levels significantly increased (Brinkworth et al., 2009), whereas Bazzano et al. (2014) found no significant changes in LDL-C levels in their 59 obese participants. Brehm et al. (2009) found significant decreases in LDL-C levels in healthy obese women at interim point of a 6-months trial and Westman et al. (2002) found that over a 6-months period LDL-C levels decreased significantly in healthy overweight and obese men.

In the SR and MA conducted by Santos et al. (2012) (see 3.4.1.3) the team calculated that initially there were no significant changes in LDL-C concentrations but that in studies lasting between 12 and 23 months or lasting >24 months decreases in LDL-C levels were observed. However, Santos et al. (2012) defined LC as <40%E and do not differentiate between different categories of LC diets. This limits the applicability of their findings to the present study. More recently Gjuladin-Hellon et al. (2018) did differentiate in their systematic review and meta-analysis of eight RCT with >100 participants (overweight/obese and healthy) between four categories of reduced CHO intake. They found that at six, 12 and 24 months there were actually no significant changes in LDL-C levels compared to the LF controls, although concentrations increased. Whether the prescribed diet was a KD or a MCD made no difference. However, adherence to the diets was not reported, although the authors noted that adherence decreased after six months. It can therefore not be excluded that CHO

liberalisation might have taken place in the longer studies. Like the present study, all diets were ad libitum.

The present study was of short duration (8 weeks) and it can therefore only be speculated whether the overall significant increases in LDL-C concentrations under LCHF conditions would have eventually become non-significant like in the meta-analyses by Santos et al. (2012) and Gjuladin-Hellon et al. (2018) on medium- to longer-term RCTs.

A number of researchers have highlighted that there was a considerate amount of inter-individual variability regarding changes in LDL-C levels in response to a LCHF diet. In a study conducted by Sharman et al. (2002) of normal-weight men 33% of individuals presented with decreases in the LDL-C levels and 58% of individuals with increases. The increases could be as high as 70% from baseline levels. Tay et al. (2008) randomised 45 abdominally obese adults to an energy-deficient LCHF for six months. Overall, participants' LDL-C levels remained unchanged. However nearly a quarter of participants presented with an increase of ≥10%. The team also observed that there was a different response in males and females on the diet. In men, LDL-C levels tended to increase, whereas in women they decreased. This led the authors to hypothesise that responses and sensitivity levels to increased SFA intake as common in LCHF diet might be individual. Similar findings were made by Yancy et al. (2004) in their 6-month trial. A couple of participants had to drop out when their LDL-C levels increased substantially after 3 months. Thirty percent of participants with available baseline and endpoint data increased their LDL-C levels by >10%. Westman et al. (2002) found that in their 6-months trial 71% of the participants presented with a decrease and 12% with an increase in LDL-C levels. Therefore, an extended look at the impact on LDL-C concentrations in the participants of the present study was warranted. In fact, two of the participants in the LCHF group defied the assumptions about LCHF diets and LDL-C concentrations by presenting with decreased concentrations by study endpoint.

One of the arguments against LCHF diets is amount of SFA that tends to be consumed with it (Noakes and Windt, 2017; Volek et al., 2004). The investigation into whether there are inter-variable responses of LDL-C concentrations to LCHF diets high in SFA was therefore taken a step further by examining whether high SFA intake non-discriminatorily results in high LDL-C concentrations. Indeed when individual participants consumed what turned out to be their maximum intake of total SFA over the course of the intervention 60% actually presented with their lowest LDL-C concentrations. The other 40% did seem to follow the assumed pattern that SFAs increase LDL-C concentrations. This might have also been a sign

that for some participants SFAs were used as energy substrates and therefore no longer downregulated LDLR and affected LDL-C clearance, whilst in other CHO was still the preferred energy substrate and SFAs remained in the circulation for longer.

In the majority of studies the LCHF diet prescribed is the Atkins diet. Volek and colleagues (2000) advised their participants to consume mainly lean beef, poultry, fish, canola and olive oil, nuts and seeds, peanut butter, egg substitute (equivalent to one egg per day) and protein powder. They were asked to consume only moderate amounts of vegetables and hard cheese but to avoid most other dairy products. They also took a daily fish oil supplement and fibre supplement. Similar to findings in the present study Volek et al. (2000) found that overall LDL-C significantly increased, with four participants showing decreases and six participants showing increases. In a randomised trial reported by Veum and co-workers (2017) 20 overweight and obese men presenting with abdominal obesity were instructed to consume two meals containing fish per week, to have vegetables with every meal and to limit intake of plant oil containing high amounts of n-6 fatty acids. Main sources of dietary fat recorded by participants were butter (with 70g/d by far the largest contributor), cream, nuts/seeds, sour cream, olive oil, coconut milk, mayonnaise, coconut oil, avocados and olives. Meat, eggs, fish, cheese and legumes were listed as main contributors to protein intake; however, the majority of these also contains considerable amounts of fat. In the majority of participants LDL-C levels initially increased and then decreased again but these findings were not significant over the course of the study. Whilst Veum and colleagues (2017) supplied details of their participants' actual food consumption, to the candidate's knowledge none of the other LCHF trials did so. However, Veum et al. (2017) also did not investigate the potential impacts of the actual dietary composition further.

Four participants in the EWG group, which overall followed a moderate-CHO diet (see chapter 3.6.1.3), also presented with increased LDL-C concentrations at endpoint. However, when looking at the time point when all participants in the EWG group consumed their maximum CHOs, four of these presented with their lowest LDL-C levels. Maximum CHO intake ranged from 48%TE to 58%TE. The greatest contributors to CHO intake at this point were white grains and cereals and fruit and vegetables. An inverse association between fruit and vegetable intake and LDL-C concentrations has been observed in population-based cross-sectional studies (Djoussé et al.; 2004; Mirmiran et al., 2009). Intake of wholegrains, which is recommended in the UK dietary guidelines, was limited in the present sample, even with maximum CHO intake, despite being advised to increase intake. Wholegrains have been associated with improved LDL-C concentrations. However, although a meta-analysis and systematic review by Hollænder et al. (2015) confirmed this, once wholegrain oats were

removed from the analysis, the superior effect of total wholegrains was no longer present. A set of systematic reviews conducted for the SACN report on dietary CHO (2015) also confirmed that there was adequate evidence for oat bran and β-glucans to lower LDL-C concentrations but no effect was observed for total carbohydrates or sugar. That the highest levels of CHO intake coincided with the lowest levels of LDL-C in the EWG participants would appear to have beneficial CM effects in the light of LDL-C being a primary target in primary and secondary CVD prevention.

An important factor to take into consideration in the context of atherogenicity of lipids is the discordance between LDL-C concentrations, concentrations of subfractions of LDL-C and LDL particle size to more precisely assess CM risk (see chapter 3.1.2.2). Whilst this study did not assess the number of LDL particles and their subclasses, the concentration of sdLDL-C was measured. Regardless of whether LDL-C concentrations increased or decreased in participants in all participants in the LCHF group sdLDL-C concentrations decreased, lowering CM risk for this group. These findings are accordance with those made by previous LCHF studies that reported a decrease in sdLDL-C concentrations or an increase in LDL particle size (see chapter 3.1.2.2). In the majority of participants, intake of (full-fat) dairy and dairy products increased a lot. Sjogren et al. (2004) found beneficial effects of milk-derived fatty acids on LDL-C particle size distribution and concluded that even small increases in consumption of these could potentially decrease CM risk by up to 38%. They hypothesised that the short- and medium-chain SFA in milk were preferentially metabolised compared to longer-chain SFAs exerting beneficial effects. This would especially occur if dairy products dominate the dietary pattern as was the case in the present study when total SFA intake was at its maximum. Raziani et al. (2018) found that consuming full-fat versus reduced fat cheese over a 12-week period significantly reduced sdLD-C concentrations in abdominally obese men but not in women. Siri-Tarino et al. (2015) reported in their systematic review that the consumption of dairy products accompanied by high levels of lean beef seemed to have detrimental effects on sdLDL-C concentrations and concluded that the interaction between beef and dairy, a mix of sources, might be atherogenic.

In all but one participants in the EWG group sdLDL-C levels increased resulting in a potentially detrimental CM risk profile. This occurred even within the context of a moderate CHO diet and without exceeding SFA recommendations. These findings would be in line with the observations made by Siri-Tarino et al. (2010) that even moderate CHO intake could lead to an increase in sdLDL-C levels.

There was a significant effect of time for TG. Triglyceride concentrations decreased by 27% in the LCHF group and also decreased in the EWG group but to a smaller magnitude. Reflecting this, the TG/HDL ratio decreased for the majority of participants in both study arms, and more so but not significantly in the LCHF group. This is in accordance with previous LCHF studies (see chapter 3.4.1). The reduction in TG in the LCHF group is one of the reasons why proponents of LCHF diets argue that they are beneficial for CM health (Noakes and Windt, 2017; Volek and Phinney, 2011). The findings from the present study reflect previous findings that TG levels are generally and consistently decreased by a LCHF diet (both ketogenic and non-ketogenic) independent of study duration or study population (Brehm et al., 2009; Brinkwort et al., 2009; Sharman et al., 2002; Tay et al., 2008; Veum et al., 2017; Volek et al., 2009; Westman et al., 2002; Wood et al., 2006) and that decreases are most dramatic in those with higher baseline levels (Volek et al, 2005).

As with LDL-C, SFA from different food sources also seem to have differential effects on the level of change in TG levels. Due to the shift to dairy products by the LCHF participants, the effects of dairy products on TG are of particular interest in this context. Drouin-Chartier et al. (2016) concluded in their review that dairy products overall had a neutral effect on plasma triglyceride concentrations, although this could only be based on a small number of trials. A meta-analysis by de Goede et al. (2015) of five cross-over trials comparing the impact of butter versus cheese consumption on triglyceride concentrations in adults found no significant differences between the two. Two cross-over trials comparing milk with cheese (de Goede et al., 2015) were also reviewed. One did not find any significant differences in TG concentrations; the other found that milk non-significantly increased TG levels compared to cheese. A systematic review and network meta-analysis of 66 randomised trials including 3595 adults with or without diabetes and lasting ≥4weeks found red meat to be one of the most effective foods to reduce TG concentrations (Schwingshackl et al., 2018). Unlike the present study no LCHF trials have looked at the contribution that different SFA- rich food sources and different subtypes of SFA made to the diet's composition to consider potential atherogenic effects of these.

Overall, TG concentrations decreased in the EWG arm. This might reflect that average CHO intake was moderate rather than high. A recent study comparing a HC DASH diet (55%TE), a high-fat diet containing 43%TE CHO and a control diet containing 47%TE resulted in the highest triglyceride concentrations in the DASH group (Chiu et al., 2016). The overall and individual amounts of CHO consumed by all participants but one during the intervention were below the levels of 55%TE that Parks (2001) noted as the threshold for CHO to induce

hypertrigleridaemia. Two participants presented with increased TG concentrations by endpoint but not at levels considered hypertriglyceridaemic.

#### 3.7.2.2 The impact of the intervention on fasting plasma insulin concentrations and insulin resistance status

Proponents of the LCHF diet argue that one of its benefits is the reduction in circulating plasma insulin levels and improvements in IR (Noakes and Windt, 2017; Volek and Phinney, 2011). Other note concerns that high-fat diets, in particular those high in SFA, cause or increase IR (MacDonald, 2016; O'Connor and Rudkowska, 2019; Volek et al., 2004). There was a significant effect of time and diet*time interaction for fasting plasma insulin and HOMA2-IR. Both groups presented with decreased insulin concentrations and decreased HOMA2-IR but the changes were greater in the LCHF group. A significant decrease in fasting plasma insulin concentrations and IR, which occurred in the present study, is a common outcome in LCHF trials (see chapter 3.1.2.3). This occurred even with high SFA intake.

#### 3.7.2.3 The impact of the intervention on adipokine and hepatokine concentrations

##### 3.7.2.3.1 Adiponectin

Circulating adiponectin is inversely associated with VAT, shown to be detrimental to CM health (see chapter 3.1.3). Low or decreasing adiponectin levels are therefore an indicator of dysfunctional adipose tissue and consequently that an individual's risk of CM might have increased. This means that in the present study the decreases in adiponectin in the LCHF and the increases in the EWG group would signal detrimental effects for the LCHF group and be beneficial for the EWG group. It might point to changes in VAT volumes. Weight loss (i.e. reduction in fat mass) has been found to be one of the most effective ways to increase adiponectin concentrations (Pischon and Rimm, 2006).

The positive impact on adiponectin concentrations in previous LCHF trials (see chapter 3.1.2.4.1) was not replicated in the present study. Although the decrease in adiponectin concentrations was not high in the LCHF arm, it was present nonetheless and all participants with complete data presented with decreases by study endpoint. It is unclear whether the composition of the LCHF diet in the present study had an impact on adiponectin concentrations due to for example the increased intake in dairy. Van Loan and colleagues (2011) implemented a 12-week controlled feeding study, which included 35 adults randomised to consume ≤4 servings per day of dairy. There were no significant differences over time for adiponectin concentrations.

In the EWG arm adiponectin concentrations increased slightly over the course of the intervention, which is a beneficial development in terms of CM risk. Concentrations were at their minimum in three participants when CHO intake was also at its minimum, which means when intake could be classed as moderate. Paniagua et al. (2007) conducted a randomised cross-over trial with 11 overweight/obese and abdominally obese adults following a HC (65%E), MUFA-rich (23%E) and SFA-rich (23%E) for 28 days each. The CHO-rich diet resulted in the highest adiponectin concentrations at endpoint. Song et al. (2016) however, found that in a randomised controlled feeding study a moderate-CHO, moderate-fat (with intakes similar to the actual intake of the present sample) adiponectin concentrations significantly decreased after six weeks. A low-fat, high-CHO diet demonstrated even higher decreases. Qi et al. (2005) observed in a cross-sectional sample of 780 diabetic men that those with the highest consumption of cereal fibre had significantly higher adiponectin concentrations compared to the group with the lowest intake. Wholegrain consumption has also been positively associated with adiponectin concentrations (AlEssa et al., 2016), However, it is not clear how much these findings would be corroborated by the present sample as wholegrain consumption by the participants was limited.

#### 3.7.2.3.2 Leptin

In the present sample the LCHF diet and the EWG diet had opposite effects on leptin concentrations. They decreased significantly in the LCHF group and increased significantly in the EWG group. As leptin is derived from adipocytes (Mechanick et al., 2018) whether these changes occurred at the same time as changes in adipose tissue was investigated as part of this present study. The decreases in the LCHF group corroborate findings form previous studies that also observed significant decreases amongst their cohorts (see chapter 3.4.1). Unlike in the present study where the decreases could only be observed in the male LCHF participants, these other studies investigated either mixed cohorts or women only cohorts. Leptin concentrations were at their highest levels with minimum total SFA intake, which was also the time point with the highest CHO intake in all participants. A high-fat, high total SFA diet therefore appeared to have beneficial in this male sample.

The 12-week controlled feeding study implemented by Van Loan and colleagues (2011) in 35 adults randomised to consume ≤4 servings per day of dairy also assessed leptin concentrations. There were no significant differences over time for leptin. Concentrations decreased but standard variation of the mean was high. Most participants in the present study decreased their consumption of meat and processed meats in favour of dairy products. When Chai et al. (2017) investigated a sample of 312 men they found that over a nine-year period

leptin concentrations were significantly and positively associated with baseline red and processed meat intake.

In the present sample, the participants in the EWG arm increased their leptin concentrations. Song et al. (2016) however, found that in a randomised controlled feeding study a moderate-CHO, moderate-fat (with intakes similar to the actual intake of the present sample) decreased leptin concentrations significantly. Their study did not measure fasting plasma insulin concentrations.

#### 3.7.2.3.3 Fibroblast growth factor 21

The results from the current study showed that higher CHO intake resulted in higher FGF21 levels, whereas reduced CHO intake had the opposite effect. These findings re-iterate Solon-Biet et al. (2016) conclusions that in the presence of sufficient protein intake CHOs seem to be the main driver of FGF21 concentrations. The present study was the first study to investigate the impact of a prescribed EWG diet on FGF21 concentrations and to the candiate's knowledge no other studies have examind the impact of following any types of dietary guidelines on these levels.

### 3.7.2.4 The impact of the intervention on systolic and diastolic blood pressure

This study found that both systolic and diastolic blood pressure were significantly reduced with the LCHF diet but not with the EWG diet. The findings for the LCHF group reflect the results from previous studies that consistently observed significant decreases in both blood pressure measurements. This was regardless of the sample population being male (Bradley et al., 2009; Phillips et al., 2008; Westman et al., 2002) or a mixed cohort (Foster et al., 2010; Tay et al., 2008; Yancy et al., 2004), normal-weight, overweight and/or obese (Bradley et al., 2009; Foster et al., 2010; Tay et al., 2008; Westman et al., 2002), healthy or presenting with metabolic abnormalities (Tay et al., 2008; Yancy et al., 2004). Santos et al. (2012) calculated in their meta-analysis that LCHF diets tended to significantly decrease both systolic and diastolic blood pressure by 3.1 mmHG and 4.8 mmHG respectively. Bradley et al (2009) also reported significant decreases in blood pressure after 12 weeks intervention but highlighted that all participants had been normotensive at baseline. Meckling et al. (2002) found that 43% of participants who had been hypertensive at baseline presented with normal BP at endpoint. The changes in the present sample were even more beneficial with 75% of LCHF participants who presented with systolic and/or diastolic hypertension at baseline became normotensive at endpoint. The study duration also made no difference in overall findings of a decrease in blood pressure with the exception of the one-year trial conducted by Bazzano et al. (2014), in which reductions were non-significant.

When total SFA intake was at its minimum in the LCHF arm and less SFA were derived from dairy both SBP and DBP were at its maximum levels in nearly all participants. A number of studies have associated the intake of either full-fat or low-fat dairy with improved BP and reduced risk of hypertension, although it seems to be less clear whether SFA actually negates some of the potential positive impacts or exerts a neutral effect (Nestel, 2019; O'Connor and Rudkowska, 2019; Scavuzzi et al., 2017). In their recent meta-analysis Schwingshackl et al. (2017) found no difference in effect between high-fat and low-fat dairy products on BP. In the present sample, those consuming high levels of SFA did not present with increases in BP.

Maximum CHO intake had more pronounced effects in the participants of the EWG arm and resulted in peak SBP for two participants and minimum SBP for three participants. Findings for DBP were similar with 80% of participants presenting with either maximum or minimum measurements at this point of dietary CHO intake. However, there was no clear CHO threshold that determined whether BP was minimum or maximum. Interestingly, the majority of participants with complete data points increased overall BP whilst decreasing CHO intake. Considering that the LCHF arm presented with improved BP with reduction in CHO intake this begs the question whether other dietary factors rather than the CHO contained in foods impacts BP. Schwingshackl et al. (2018a) found in their meta-analysis that fruit and vegetables, legumes and wholegrains were the best food groups to lower BP. Refined grains faired less positively in their analysis but dairy was not analysed for impact on BP. Although these are all CHO-rich food groups other nutrients contained within their food matrix might therefore be far more influential. The DASH diet (Dietary Approaches to Stop Hypertension), which has consistently been found to be beneficial for BP (Ozemek et al., 2018; Saneei et al., 2014; Schwingshackl et al., 2018b), emphasises the intake of fruit, vegetables and wholegrains but also low-fat dairy. Although it is classed as a HC diet, it is also rich in other nutrients that might play a confounding role.

### 3.7.3 Body composition

Total and depot-specific AT is associated with increased CM risk and therefore an important outcome measures in any intervention assessing the impact of diet on CM risk factors. Changes in weight status seem to be a common outcome in studies investigating LCHF diets and changes in FM are often reported. Contrary, WC and VAT are less frequently investigated adiposity proxies in this field. As hypothesised compared to the LCHF group presented with significantly greater decreases in any of the adiposity proxies presented in this chapter. This is in accordance with the results from previous studies, which have

consistently come to the same conclusion, regardless of adiposity status of the sample population. The participants in the present study were nearly all overweight and the reduction in adiposity proxies seems to be independent of BMI status.

Volek et al. (2002) demonstrated that these changes occurred even in normal-weight healthy individuals on a LCHF diet. When looking at individuals in each study arm, this was also confirmed for every single individual allocated to the LCHF diet. In accordance with other studies (see chapter 3.1.3) the EWG group (in other studies mainly LF group) also presented with reductions in three out of the four adiposity proxies presented in the chapter. But overall FM (%) increased in this group. The control groups in previous studies were prescribed a LF diet, whereas the majority of the EWG participants in the present study consumed total fat 30%TE. Rather than experiencing a large reduction in adiposity between baseline and interim point, the reductions in the LCHF group occurred at a steady pace. As this was a fairly short intervention it is impossible to tell whether adiposity proxies would have continued to decrease at the same pace.

Thus far there have not been many LCHF trials that reported on the effect of the diet on VAT (see chapter 3.1.3). In contrast to the present study no significant changes in VAT compared to the control group were observed (Gepner et al., 2018; Veum et al., 2017).

No previously conducted LCHF studies have considered NC as a CM risk factor. Neck circumference has been shown to be correlated with VAT (Ataie-Jafari et al., 2018; Yang et al., 2010) and like VAT, NC decreased in the LCHF group and to a greater magnitude than in the EWG group, but effect of diet was not significant. This might have been due to the relatively short study duration and the small sample size. To the candidate's knowledge the CALIBER study is not only the first study to assess changes in NC when following a LCHF diet, but research investigating the impact of any type of dietary intake seems to be scarce indeed. One study assessed the effect of following a six-months LF diet (<25% total fat, <7% SFA) on dyslipidaemia in 59 liver transplant patients (Pinto et al., 2016). Participants in this trial were classed as overweight with NC of >37cm for men and >34cm for women and as obese with NC of >39.5cm for men and >36.5cm for women. There was a significant reduction in NC in men and a non-significant increase in NC in women between baseline and endpoint. However, dietary adherence was not reported in this trial.

The greater reduction in all adiposity proxies in the LCHF group is most likely also due to increased ß-oxidation to switch from CHO to fat as fuel and increased lipolysis (Volek et al., 2008).

### 3.7.4 Physical activity

Both groups exceeded the physical activity recommendations for MVPA during the intervention but had already reported high habitual PA levels for the month preceding the study. That self-reported levels (and significantly so in the LCHF group) were lower than objectively assessed baseline levels might be due to the assessment tool used and the data processing methods applied to the raw accelerometer data. Many physical activity questionnaires tend to provide an under-estimate of activity (Neilson et al., 2008), although the RPAQ has demonstrated to have good validity with VPA (Besson et al., 2010). Participants had been asked not to embark on any conscious change in exercise regime whilst taking part in the study, but the motivation of over-hauling their lifestyle might have been too much to resist at least at the beginning of the study. As they felt their quality of life improving (see chapter 3.6.5) they might have also felt more able to be active, which is an important potential benefit to their CM health. It has been shown that study participants tend to change their behaviour when objectively assessed (French and Sutton, 2010), which is known as the 'Hawthorne effect' (McCambridge et al., 2014), although this effect seems to be more pronounced in studies including pedometers (Bravata et al., 2007; Ewald et al., 2010).

One of the participants in the LCHF furthermore reported that their occupation involved standing and moving heavy parts. They also stated that when necessary they were working overtime. Indeed, this participant had the highest increase in MVPA levels from baseline, which might have been due to occupational commitments (which were out of their control) rather than sub-conscious increases in MVPA levels outside of working hours. Even when this participant was removed from the analysis, the LCHF group non-significantly increased their MVPA compared to baseline and was also more physically active than the EG group (data not shown).

### 3.8 Conclusion – CALIBER study

#### 3.8.1 Dietary intake, adherence and food cravings

To the candidate's knowledge, this was the first study to compare a LCHF diet with following the UK dietary guidelines. Its participants seemed to have found it easier to reduce CHO consumption considerably than to make sufficient adjustment for meeting UK dietary recommendations.

Nonetheless, overall, it seems that over the course of any KD intervention dietary adherence declines as demonstrated through self-reported (dietary assessment) or objective measures (blood or urinary ketone bodies) (Brehm et al., 2009; Hu et al., 2015; Johnston et al., 2006; Yancy et al., 2010). Specific reasons as to why adherence to ketogenic diets declines are normally not explored but only briefly reported and include intolerability of diet (Yancy et al., 2010) and not being able to comply with the diet (Tay et al., 2018). The food diaries and absence of nutritional ketosis exposed that the LCHF group in line with the literature did not fully adhere to their prescribed diet. The absence of nutritional ketosis at endpoint must lead to the conclusion that at best participants followed a non-ketogenic LC diet of <130g but >50g of CHO per day. A number of different factors might have had an impact on dietary adherence in this group. Here, practical considerations, such as the expense of the diet and the difficulties it caused when socialising, might have had an impact. On the other hand, perceived improvement in quality of life factors might have been facilitating and motivational enough to limit CHO consumption to <130 g/d. The perceived positive impact on quality of life was very strong in the LCHF group. A study undertaken by Guldbrand et al. (2014) with 30 adults with T2DM on a LC diet (CHO 20 %TE) showed that their self-assessed health scores improved across a number of domains.

Food cravings also improved in this group, especially for starchy foods, which would have also contributed to keeping CHO consumption low, albeit not sufficiently so to achieve nutritional ketosis.

Only one participant in the LCHF group stated that they had found it somewhat difficult to follow the diet and another one was not sure, whilst all the other participants (in both study groups) found it easy or very easy. Overall, it can be concluded that (perceived) lack of nutrition literacy was therefore not likely a cause for non-adherence to the diets, especially as all participants had received detailed guidelines, meal plan suggestions and stated that they had used at least some of the recipes provided. Added levels of difficulty of shopping for ingredients and cooking according to the rules would also have been a potential cause of non-adherence in only one of the LCHF participants as the majority of participants did

actually not find this any different from their pre-study lifestyle. Although 60% of participants in the LCHF group of the present study stated that the diet had been more expensive compared to pre-study, this did not cause any study attrition. However, it might have had an impact on adherence levels, as participants might have subconsciously been tempted to 'cut corners'. Raffensperger (2008) calculated the costs of the cheapest possible LC diet using linear programming and found that diets restricted in CHO were more expensive that those that contained the generally recommended amounts of CHO. Although the palatability of the food combinations in his model is questionable, carbohydrate-rich foods have been found to be the cheapest source of energy (Drewnowski, 2010). These findings were contradicted by participants in a study undertaken by Guldbrand et al. (2014), who stated for their LC diet to be "cheap" (and also "tasty" and "easy to follow").

The same would apply to the impact of dealing with social eating occasions outside the home as all participants in the LCHF group found this to be somewhat harder or a lot harder. Booth et al. (2013) conducted focus groups with T2DM patients and health care providers on barriers to changing eating habits. Participants stated that social occasions were particularly difficult due to the lack of appropriate food items. Although the LCHF group had been given guidelines on how to deal with social situations, in practice these might have been hard to follow due to the lack of control and potential worry of offending the host. In contrast, in their home environment was a level of control that participants could exercise, so that lack of adherence would unlikely have occurred consciously. The practicalities of following a LCHF diet could have decreased adherence levels more in the LCHF group than in the comparison group.

For LC diets adherence can be further impacted by the regimen's potential short-term side effects, such as the 'keto-flu' (including headache and general weakness), impaired cognition, gastrointestinal upsets such as constipation or diarrhoea, muscle cramps. This can potentially cause attrition in studies as a consequence (Crowe, 2006; Harvey et al., 2018; Westman et al., 2007; Yancy et al., 2004). Although there were a few occurrences of adverse events reported by participants in the LCHF group, this did not cause any dropouts and participants did not report to increase their CHO intake to alleviate side effects.

As demonstrated by analysis of their FDs, the EWG group as a whole followed a moderate-CHO diet. Adherence to dietary guidelines and healthy eating has been linked with a variety of facilitators and barriers (Carrara and Schulz, 2018; Kelly et al., 2016; Nicklas et al., 2013). These include age, educational, conjugal and socioeconomic status. They are also associated with other factors, such as nutrition knowledge (Bonaccio et al., 2013), and food and

nutrition literacy (Gibbs, 2017; Palumbo, 2016; Poelman et al., 2018; Yuen et al., 2018). In addition, confidence in food skills of both study participants and households' main food providers or 'household gatekeepers' can also play an important role (Burton et al., 2016). The economic costs of adhering to dietary guidelines have been explored in a number of studies. More recently Jones et al. (2017) calculated that following UK dietary recommendations as outlined by SACN were up to 29% more expensive than an isoenergetic unhealthy diet. In a similar analysis, Mulik and Haynes-Maslow (2017) found that the adhering to US dietary recommendations might be prohibitively expensive for low-income families. Zorbas et al. (2018) conducted a systematic review of 39 qualitative studies exploring the factors that individuals perceive to influence adherence to healthy eating (as defined by national dietary guidelines). They confirmed the factors named above and additionally identified taste, habits, marketing and media, food availability and affordability, convenience and time and the built environment as potential barriers to following dietary recommendations.

The design of the present study attempted to pre-empt some of the known barriers to dietary adherence. This occurred by providing detailed guidelines on the allocated diets (food and nutrition knowledge), 24 hour access via telephone or email for any queries (nutrition support and education). Meal plan suggestions (where feasible within the context of the allocated diet) were matched to food and taste preferences and time available for cooking. In addition, the recipes provided to the participants matched the number of people in the household. Consideration was also given to the household gatekeepers (family support).

For the EWG group there were hardly any reported negative practical implications of being assigned to this study arm, but perceived quality of life was no different compared to pre-study experiences, therefore lacking any motivational factors. Overall, it seemed to have been more difficult for the group overall to increase CHO intake sufficiently. This might be partly due to the quality of CHO sources consumed not to be sufficiently different from baseline levels. Future studies should therefore consider more practical, hands-on educational and more intensive and more finely-tuned measures to adjust dietary intake. This could be for example by demonstrating to individuals how meals that they might typically consume could be adjusted to increase CHO content.

The only food craving that showed significant decreases over time was that for fatty foods. Mean scores decreased more in the LCHF than in the EWG group, which was most likely due to the increase of total fat consumption within the remit of the LCHF diet. Interestingly, again at interim point when CHO intake was at its lowest level, participants in the LCHF group were on average less inclined to give in to cravings for fatty foods and found it the

least difficult to resist than at any other point. This might have also been an indication of the satiating effects of a KD.

In the EWG group cravings for fatty foods were at their lowest when CHO intake (%TE) was at its highest (and total fat intake (%TE) was at its lowest levels). At this point the group also felt that it was easiest to resist cravings and that it was less difficult to resist cravings for fatty foods compared to endpoint. It is not clear why both the LCHF and EWG had similar results regarding cravings for fatty foods at the same time point when their dietary intakes of CHO (%TE) were at opposite ends. Potentially psychological aspects of 'being on a diet' played a role. Additionally, interim point measurements took place during the height of summer and rising temperatures might have decreased appetite (Herman, 1993; Vincent et al., 2018). This might have had a confounding effect on cravings for other food categories on the FCI-UK, too.

Interestingly, the EWG group reported that their cravings for sugary and fatty foods were at their lowest at interim point when CHO intake (%TE) was at its highest level. Participants also reported to give the least into temptation for these food types and found it the least difficult to resist temptation. Cravings for starchy foods however, were at their highest at interim point when CHO consumption (%TE) was at its highest level. Although, participants had been encouraged to consume high amounts of CHO in line with the dietary guidelines at this point they firstly reported to give in the least to consuming these foods and secondly that it was the least difficult to resist temptation. By endpoint cravings for starchy foods had increased beyond baseline levels and participants reported to give in slightly more to temptation but found this increasingly difficult. The lack of adherence to CHO intake as per dietary guidelines is surprising in this context and the reasons for this are unknown. There might be a possibility that participants were slightly influenced by the nature of the study, which also included a LC option. Maybe this led to the subconscious perception that CHO were 'bad' and intake should be restricted after all, especially potatoes, bread and pasta, which are some of the starchy foods included on the FCI-UK. In addition, following dietary guidelines participants had been encouraged to choose wholegrain varieties whenever possible, and a reluctance to changing dietary habits in this context and giving up white bread, rice and pasta might have led to these foods not being consumed as much in the first place.

### 3.8.2 Clinical markers of cardiometabolic risk

The present study corroborated a number of findings from previous studies where a LCHF diet prescription was linked to overall significantly increased LDL-C, decreased sdLDL-C and decreased TG concentrations, improved TG/HDL-C ratio, decreased plasma insulin concentrations and HOMA2-IR and decreased systolic, and diastolic blood pressure. Findings for the EWG arm of this study were potentially less unambiguous than expected for HC diets, which was probably attributable to the fact that the majority of participants did not actually follow a HC diet.

These findings are also an interesting reflection of the initial secondary analysis of the NDNS RP 2008/09 – 2013/14 that provided the rationale for the CALIBER study. Although the individuals assigned to the lowest quartile of CHO consumption or the highest quartile of SFA consumption did not overall present with dietary extremes (as prescribed to the LCHF arm of the present study), the odds of presenting with clusters of CM risk as expressed through the definition of MetS were lower in these groups compared to those in the highest CHO and lowest SFA intakes. The definition of MetS includes markers which were examined in more depth in the participants of the present study, such as DBP, GLUC, SBP, TG and WC. Therefore, both the findings from study 1 and study 2 of this thesis present a combined view of potential benefits of reduced CHO intake and minimal risk of increased SFA intake. As the secondary analysis of the NDNS RP only investigated clusters of CM risk, rather than the individual components of MetS, it it not possible to comment on the applicability of the findings made for individual risk markers examined in the CALIBER study. Further in-depth secondary analysis of the NDNS RP 2008/09 – 2013/14 and future intervention studies are needed to corraborate these findings.

For both diet arms there was a high degree of inter-individual variations, especially for LDL-C, which has previously been highlighted in other LCHF studies. Hardly any LCHF studies have reported on the actual dietary intake by their participants and have failed to acknowledge that similar to other (HC) dietary patterns the food sources and different subgroups of saturated fatty acids are likely to exert differential effects on markers of CM risk.

This present study was the first one to investigate which SFA-rich food groups and associated subtypes of even-chain SFA LCHF contributed to participants' dietary intake to consider potential atherogenic effects of these. Furthermore, it differentiated for both diet groups how the two prescribed diets affected surrogate risk markers on an individual basis. This was in recognition that nutrition science needs to consider inter-individual

physiological differences impacting responses to dietary intake and take a more personalised approach.

In the present study, when participants' SFA intake was at its lowest level in 80% of the participants the by far greatest contributors to total SFA were meat and meat products. All participants in the LCHF arm derived the majority of their total SFA from dairy and meat products when their total SFA intake was at its maximum. This means that to increase SFA intake dietary patterns were adjusted to include a far larger amount of dairy products (mainly cheese and cream), rather than for example simply increasing meat intake. For the majority of participants in this group this was also reflected in the dietary saturated fatty acids profile, where both intake of palmitic and stearic acid decreased but increased for the other even-chained SFAs. Whilst stearic acid is considered to have a neutral effect on LDL-C concentrations, palmitic acid seems to increase it (Fernandez and West, 2005).

Interestingly, in the two participants who presented with the highest LDL-C levels when SFA levels were at their maximum, intake of lauric acid (found in coconut oil, but also in milk) decreased, whereas intake increased in two of the participants presenting with the lowest LDL-C levels at this point. High-fat dairy products tend to increase LDL-C concentrations (Thorning et al., 2016), which was supported by the overall findings by the present study. However, Thorning et al. (2016) highlighted that an increase in LDL-C levels was simultaneously attenuated due to the minerals (calcium) found in dairy products exerting beneficial effects.

Under standard dietary conditions, where CHO is available in abundance to supply the body with energy, SFA downregulates the LDLR, which means that LDL clearance from the circulation is reduced and consequently LDL-C concentrations increase (Fernandez and West, 2005; Mustad et al., 1997; Woollett et al., 1992). When CHO intake is severely restricted, the body increases ß-oxidation to derive energy from fatty acids. Under these circumstances SFA get cleared out of the circulatory system quicker (Volek et al., 2008) and therefore less SFA remains to downregulate LDLR. This has previously been demonstrated through studies analysing the amount of plasma SFA, which is reduced under conditions of CHO-restriction (Forsythe et al., 2008; Forsythe et al., 2010). Those LCHF participants that presented with their lowest LDL-C concentrations when SFA consumption was at its highest might have therefore used sufficient amounts of SFA as energy substrate to prevent downregulation of LDLR. Consequently, this would have meant that LDL-C was cleared from the circulation and concentrations decreased.

In all but one participant in the EWG group sdLDL-C levels increased resulting in a potentially detrimental CM risk profile. Although these increases coincided with minimum

CHO consumption in 75% of these participants, at this point fructose consumption exceeded the levels of when total CHO was maximal. The majority of fructose was derived from fruit (and vegetables) and it would therefore have to be considered how much the perceived benefits of consuming these foods are being outweighed by the detrimental effect on the participants' atherogenic profile. Increase in fructose intake leads to increased lipogenesis and fructose is more lipogenic than glucose (Volek et al., 2008). This leads to an over-production of large, TG-enriched VLDL particles in the liver, which not only stay in the circulation for longer but also lead to an increased production of sdLDL (Griffin, 1999). These mechanisms most likely explain the increase in sdLDL-C concentrations in the EWG group, even when CHO intake was more moderate.

Changes in TG levels in the present study ranged from -46% to 16% with the greatest changes in those participants with the highest baseline levels. One of the confounding factors of this might have been a decrease in VAT (see chapter 3.6.8.1). A decrease in VAT would generally lead to a decrease in TG concentrations as less substrate (free fatty acids) would be available from the adipocytes that would be converted into triglyceride-rich lipoproteins in the liver (Björnson et al., 2016; Chan et al., 2004). Furthermore, a reduction in CHO downregulates the process of lipogenesis (due to reduced insulin concentrations) and leads to a reduction in hepatic TG-rich particles. It is for this reason that hypertriglyceridaemia is often accompanied by elevated sdLDL-C concentrations (Cho et al., 2015).

In 60% of participants when total SFA intake was at its maximum, insulin concentrations were at their lowest point. The decrease in insulin concentrations is a response to the decrease in CHO, whilst the body kept plasma glucose concentrations stable. In this turn resulted in lower HOMA2-IR values. Doubts have been expressed whether this can be sustained in the long-term (Kosinski and Jornayvaz, 2017) and indeed further studies are needed to confirm whether this would be the case. Nonetheless, at endpoint all participants presented with improved insulin concentrations and HOMA2-IR status.

Whilst in the LCHF group all participants decreased insulin and HOMA2-IR, in the EWG group two participants presented with increases in both at study endpoint. These coincided with a decrease in CHO intake. Sixty percent of participants presented with their lowest insulin concentrations and HOMA2-IR values when CHO intake was at a maximum, which was in all cases ≥50%TE. One of the models attempting to explain the effects of CHO on insulin concentrations is the *carbohydrate-insulin model* (CIM) of obesity (Ludwig and Ebbeling, 2018). Foundation of this model is the assumption that processed CHO, which are quickly metabolised (high-glycaemic CHO) drive hyperinsulaemia. Although the authors

emphasise that it is processed CHO driving this mechanism, they also premise (and probably not wrongly) that a high-CHO, Western diet contains a lot of the processed CHOs (Ludwig and Ebbeling, 2018). Ludwig (2018) highlights the benefits of wholegrain and low-glycaemic foods. Under these conditions a LCHF diet, which by definition does indeed cut out all the processed CHOs (due to intense CHO-restriction), would indeed result in lower insulin concentrations as with the present sample. However, the findings from the EWG group showed that despite significantly higher CHO intake some participants also presented with lower insulin concentrations. This might reflect an improvement it diet quality (i.e. limited amounts of processed CHO), but the more detailed analysis of the intake of CHO-rich foods showed that one of the main staples of their diet were white starchy foods. Ludwig and Ebbeling (2018) acknowledge that there might be other factors, including physical activity, sleep, that might affect insulin secretion, but the magnitude of these interactions is difficult to verify.

In the context of leptin secretion and concentrations the effects of circulating insulin also needs to be taken into account. Insulin stimulates the secretion and production of leptin in AT via the PI3K-PKBmTOR Pathway. Whether there are other pathways executing this function is currently not known (Leyva et al., 1998; Tsai et al., 2012). There were significant decreases in fasting plasma insulin concentrations, which are common in LCHF diets due to the reduced CHO intake. Consequently, it could be speculated that the decrease in insulin resulted in a downregulation of leptin expression. Interestingly, in a recent murine model Perry et al. (2018) found that the switch from fuel-utilisation from carbohydrate to fat-oxidation occurs when both insulin and leptin concentrations are sufficiently decreased. Future research needs to establish whether these findings are also applicable in humans to further elucidate the mechanisms of LCHF diets and their potential benefits.

Although insulin concentrations also decreased significantly in the EWG sample of the present study this did not translate into decreased into reduced leptin concentrations like in the LCHF group. Other mechanisms associated with leptin secretion might therefore have taken precedence. An increase in fat mass in EWG participants (see chapter 3.6.8.1) might have resulted in higher circulating leptin concentrations regardless of a decrease in insulin concentrations. As leptin is adipocyte-derived the decrease in both FM and VAT in the LCHF group and the increase in FM in the EWG group could therefore be underlying factors for these findings. The greater magnitude of change in circulating leptin concentrations in the LCHF group could be attributed to the reductions in both FM and VAT.

In this context it is therefore surprising that adiponectin concentrations decreased in the LCHF group as a reduction in AT is inversely associated with increased adiponectin concentrations (see chapter 3.1.2.4.1). As the LCHF group still presented with greater adiposity than the EWG group it might be possible that the degree of AT reduction had not passed a threshold to affect adiponectin concentrations. The increase in adiponectin concentrations in the EWG group might be explained by the reduction in VAT. As adiponectin concentrations were greater and VAT volume smaller in the EWG to begin with compared to the LCHF group. These levels might have been sufficient to respond to VAT loss with increased adiponectin concentrations.

These findings also corroborated why leptin concentrations were significantly decreased in the LCHF group but did not explain the detrimental developments in adiponectin levels. More research is needed to elucidate the complex interplay between hormones affecting and being affected by macronutrient intake.

Other markers presented in this analysis, FGF21 in particular, have only recently been emerging and show a great deal of promise to elucidate differential effects of dietary intake and diet quality on CM health and disease. This hormone appears to be linked to macronutrient intake but magnitude of this and detailed mechanisms have not been fully elucidated yet. Thus far emerging evidence from experimental studies in animals and short-term trials in humans shows that whilst dietary fat does not affect FGF21 concentrations, decreased protein and under conditions of protein-restriction increased CHO intake do. With respect to protein Hill et al. (2018) argue that even if overall protein content in a HC diet is adequate, there might still be the risk of elevated FGF21 concentrations if individual amino acids are reduced. This highlights that the quality of the macronutrients consumed and the food sources play an important part in human CM health beyond the quantitative aspects of macronutrient intake. Their review paper also highlights that ketogenic diets only result in higher FGF21 levels if overall protein content is low leading to amino acid restriction. It is therefore primarily an imbalance between protein and CHO intake independent of energy intake that seems to regulate circulating FGF21 concentrations.

When the LCHF participants in the present study consumed their lowest levels of total SFA their FGF21 concentrations were at peak levels. However, under consideration of the points above this is likely to be due to the fact that this coincided with their maximum CHO intakes. At no point could protein intake be categorised as low or insufficient and therefore CHO %TE will have exerted the primary impact on FGF21 concentrations.

Two of the EWG arm participants consumed at one time point or another what would be categorised as a low-protein diet. However, the maximum intake of CHO for any of these two participants at any time point was 49.5% and minimum intake at any point was 35.3%. One participant reported protein intakes of 12.8% and 14.5% at baseline and interim point respectively and presented with the highest FGF21 levels at interim point. The other subject of interest in this context reported energy intake from protein as 14.1% at interim point but FGF21 concentrations were at actually their lowest at this point.

This might confirm that the quality of the protein supply might be paramount as these participants might have managed to achieve adequate supply of the relevant amino acids. Future research should therefore additionally consider the amino acid content of the diets consumed. This was beyond the scope of this PhD thesis.

### 3.8.3 Body composition

The findings from this current study were in accordance with previous studies that firstly a LCHF diet can decrease specific AT depots associated with increased CM risk and secondly do this at a greater magnitude that diets that are higher in CHO and lower in fat. This was the first LCHF study to investigate the impact of the diet on NC as an independent marker of CM risk. Although there was significant decrease in NC over time, this could not be attributed to any of the diets. This might have been due to the small sample size. Further investigations are required to determine whether NC is a useful surrogate marker of dietary intake. Overall, the LCHF had more beneficial effects on adiposity proxies than the CHO intake (which was on average moderate) in the EWG.

Some might argue that the LCHF group had greater reductions in adiposity because they presented with higher values for every single proxy at baseline (and indeed throughout the study) and were significantly heavier than the EWG group (see chapter 3.3.4), and that a decrease in energy would therefore contribute to quicker weight loss. However, although the LCHF group decreased their energy intake from baseline and also compared to the EWG group (see chapter 3.6.1.3) this was not significant and standard deviations for this variable were high. A confounding factor for the greater magnitude of reduction in adiposity markers could be an increase in physical activity levels (see chapters 3.6.9.2 and 3.6.9.3) to increase energy expenditure and potentially drive weight loss even further.

In the context of the CIM (see above) the reduction in CHO intake and the reduction in insulin concentrations, which occurred in the LCHF group to a greater extent that in the EWG group (chapter 3.6.1.1.2.1), might also have contributed to the decrease in fat mass

and VAT. The decrease in insulin levels would have resulted in increased lipolysis and decreased lipogenesis in the LCHF group.

### 3.8.4 Physical activity

The present study is one of the first to investigate the impact of a LCHF diet on MVPA levels in a free-living population of non-athletes. Moderate-to-vigorous PA time in the EWG group did not change much over time, which can be attributed to the fact that CHO intake did not change significantly either and remained within moderate levels. Compared to the EWG group MVPA levels in the LCHF group increased non-significantly from baseline when following a LC, but most likely not ketogenic, diet. The severe restriction in CHO did therefore not lead to reduction in higher-intensity PA. However, it is not clear whether at least some of this increase was due to being observed under study conditions (Hawthorne effect) as MVPA time recorded at baseline was significantly higher than that reported pre-study in the LCHF. Likewise, the increase in MVPA might have been the consequence of the participants reporting higher quality of life, including better sleep (chapter 3.6.5). Some argue that LCHF diets increase endurance (McSwiney et al., 2018; Paoli et al, 2015) and whilst admittedly, these conditions apply to highly-trained individuals, in a non-athlete population nonetheless switching to fat and ketones as fuel might have resulted in increased endurance and therefore increased MVPA.

That the LCHF group consistently spent more time at MVPA than the EWG group would re-enforce Phinney's (2004) statement that ketogenic diets (which utilise fat as the main fuel) do not necessitate cutting back on leisure-time or occupational physical activity. Quite the opposite, despite highly restricting CHO consumption (albeit not to ketogenic levels (chapter 2.3)) the LCHF group appeared more physically active than the EWG group, even though this was not statistically significant. Even after CHO intake increased again from interim point (chapter 3.6.1.3), fat intake remained sufficiently high and CHO intake sufficiently low in the LCHF group to have switched to utilising fat as their main fuel (Burke, 2015). It is unlikely however, that muscle tissue will have had adapted to use ketone bodies as fuel as this seems to require more trained muscle found in highly-trained athletes (Evans et al., 2017). Nearly all studies examining the impact of LCHF diets and ketone bodies on performance levels have focused on trained athletes and under exercise conditions (Burke et al., 2015; Chang et al., 2017; Evans et al., 2017), rather than in in non-athletes, or the domains of home, commute, occupational and leisure-time physical activity, as it was the case in the present sample. Contrary to the present findings, in a recent study conducted by Ebbeling et al. (2018) 42 overweight and obese participants on a 20-week LC diet (<20%)

on average hardly changed their daily MVPA time (assessed via ActiGraph GT3X accelerometers). However, dietary adherence rates were not reported. The EWG group did not change their MVPA time overall, and these findings have to be viewed in the context that this group did not increase their CHO intake sufficiently, despite being told to do so.

An important issue to consider in this context is the site on the body where the device was worn. Loprinzi and Smith (2017) assessed nine adults, who wore two ActiGraph GT9X devices simultaneously under free-living conditions on their hip and non-dominant wrist. The wrist-worn devices had significantly higher counts per minute than the hip-worn ones. However, output rates between two devices worn simultaneously on the same wrist showed significant and high correlations. Hildebrand et al. (2014) compared the output (counts) produced by two different accelerometers (including the ActiGraph GT3X+) worn on the hip and on the non-dominant wrist simultaneously in a sample of 29 adults and 29 children whilst undertaking a battery of activities ranging from lying down to running. They found that output from monitors worn on the wrist was consistently higher than from those worn on the hip, especially for more intense PA. Nonetheless, the study concluded that overall the wrist-worn ActiGraph GT3X+ performed fairly well and was therefore suitable for PA assessment, especially as wear compliance tends to be very good. Kamada et al. (2016) also found in a sample of 94 elderly women that wrist-worn ActiGraph GT3X+ devices also produced higher output than simultaneously worn hip-based devices. However, activity patterns remained consistent. This might explain why MVPA time in the present sample was overall high. However, this study used repeated measures to assess MVPA and each participant had the same accelerometer allocated to them throughout the study limiting the risk of measurement bias due to consistency in the device used. Furthermore, the focus was on potential changes in MVPA time rather than MVPA time itself, therefore the potentially high MVPA time as a result of the activity monitor being worn on the wrist can be deemed not to be a limitation in the current analysis.

The increase in MVPA time in the LCHF group in the present study was not statistically significant, which might have been due to the small sample size and the fairly short study duration. Further studies with larger samples need to be implemented to examine whether ad libitum LCHF diets in non-athletes increase MVPA, which might be initiated by decreasing weight and greater ease of moving about.

### 3.8.5 Outlook on future research needs

Building on the findings from the secondary analysis of the NDNS RP 2008/09-2013/14 that lower CHO or higher SFA intake appeared to decreased the odds of MetS, the second study

was designed as a pilot feasibility study. The CALIBER study was the first intervention that aimed to compare the effects of a prescribed LCHF and a prescribed EWG diet on a range of surrogate markers of CM risk. In addition to the components of the MetS (DBP/SBP, GLUC, HDL-C, TG, WC), it also examined the effects on markers commonly used in primary and secondary CVD prevention (LDL-C), on those that are presently being granted a more important role in this context (TG/HDL-C ratio, apoB, apoB/apoA1 ratio) and on those known to be elevated with increased CHO intake (sdLDL-C, insulin). The CALIBER study also examined the impact of the two diets on biomarkers and factors associated with dietary intake (and consequently potential CM risk), which have not been scrutinised to a great extent in this context, such as food cravings, plasma adiponectin concentrations, plasma leptin concentrations and FGF21 concentrations. Whilst FM, VAT and WC have been investigated in a number of LCHF trials, this present intervention was the first one to include NC as an additional adiposity proxy. To the candidate's awareness this is only the second study that examined the amount of MVPA undertaken by study participants at several time points throughout the intervention.

This investigation was also the first to scrutinise the intake of subtypes of even-chained SFA and food groups these were derived from in the LCHF arm. This was firstly undertaken with the awareness that different subtypes of SFA exert differential effects on surrogate biomarkers of CMD, and secondly due to the inter-individual variations in response to the diet. A few of the  overall results for both study arms seemed to confirm effects of LCHF and higher CHO diets on markers, such as LDL-C, TG and insulin concentrations. However, only by examining the effects for individual participants a far more complex picture emerged that warrants further attention in future studies. The findings also appeared to confirm that a LCHF vs a prescribed EWG diet has more beneficial effects on markers associated with atherogenic dyslipidaemia, namely sdLDL-C. Whether specific subtypes of SFA are healthier than others, or whether within the context of increased ß-oxidation and quicker plasma SFA clearance this is a minor issue, remains to be seen.

#### 3.8.5.1 Low-carbohydrate, high-fat diets in the context of more focused diet composition and synergistic effects of the food matrix

Finally, the CALIBER study was to the candidate's knowledge the first one to assess the SFA-composition of the LCHF participants' dietary intake and of food groups associated with it. Indeed, there appeared to be beneficial effects when the intake of SFA from fermented dairy, such as yoghurt and cheese (milk was discouraged in accordance with traditional LCHF diets), increased. Thus far, the prescription and analysis of LCHF diet has

taken mainly a reductionist approach by focusing on single macronutrients rather than taking the food matrix into account. This means that confounding nutrient factors, such as for example the calcium in dairy products (Alexander et al., 2016; Chen et al., 2017; Gholami et al., 2017; Peters, 2017) or the polyphenols in vegetables (Durazzo et al., 2019; Fraga et al., 2019; Mozaffarian and Wu, 2018), which have all been shown to ameliorate CM risk, are not being given sufficient consideration. Calcium can create insoluble complexes with fatty acids, thus decreasing their absorbance rates. This is turn leads to decreased cholesterol concentrations and platelet aggregation (Jolma et al., 2003). Calcium contained in the food matrix of dairy products is also thought to have hypotensive properties or to retain blood pressure homeostatis in normo-tensive individuals (Astrup et al., 2019; Markey et al., 2014). Some subgroups of polyphenic compounds have demonstrated anti-atherogenic properties by upregulating NO expression and consequently lowering blood pressure (Mozaffarian and Wu, 2018), others do so by preventing the accumulation of atherosclerotics plaques in the artery wall (Shahidi and Yeo, 2018) and by downregulating CAM (Durazzo et al., 2019). Yet others play a role in blood glucose homeostasis and reducing glucose transport in the gut (Shahidi and Yeo, 2018) and reducing IR (Fraga et al., 2019), giving them potentially anti-diabetic properties.

Future LCHF sudies should emphasise the necessity of preferably focusing on intake of (permitted) vegetables to ensure that sufficient amounts of fibre, minerals and vitamins can be consumed. The satiating effects of fats and proteins exert the benefit of reduced energy intake (Noakes and Windt, 2017), potentially leading to weight loss. However, this also carries the risk that too small amounts of vegetables are being consumed and this increases the risk of LCHF-induced micronutrient deficiencies further. One way to try and address this issue is to recommend starting with the vegetables on the plate before consuming the other food items (Flood-Obbagy and Rolls, 2009; Roe et al., 2012; Wansinck and Hanks, 2013).

Roe et al. (2012) found that consuming a portion of mixed salad (which also contained some light cheese) before the main course increased vegetable consumption by as much as 23%. In a study conducted by Wansinck and Hanks (2013) diners in a buffet line tended to fill their plates more with healthy options if these were presented first at the buffet. Regardless of which types of foods they were the research team found that nearly three-quarters of a participant's plate was filled with the first three food items they encountered at the buffet. If the foods were presented from healthiest to unhealthiest, the majority of the plate contained healthy foods and vice versa. This could have interesting and beneficial implications for

people following a LCHF diet if they are accessing nutrient-dense foods prior to focusing on the energy-rich foods on their plates.

Future studies should therefore compare the effects of LCHF diets differing in food group composition, where for example one group derives a substantial amount of SFA from meat products, the other from fermented dairy.Furthermore, the micronutrient composition of the rest of the diet also needs to be carefully scrutinised and targeted specific advice stressing the importance of the consumption of micronutrient-rich foods needs to be given. This will further elucidate which components of a LCHF diet might be more beneficial to CM health than others. This would consequently inform the design of subsequent high-quality LCHF dietary patterns to aid in the prevention and amelioration of CM risk.

As an initial starting point to aid designing this study, further secondary analysis of the NDNS should build on the research undertaken for this PhD thesis. The stratified, multi-variate analyses should be extended to elucidate the micronutrient composition and intake of foods rich in polyphenols in those quartiles of CHO, total fat and SFA intake. This could give a more detailed understanding of any confounding effects of the diet quality and more effectively inform the intervention propsed above. The individual diets rich in meat or fermented dairy could be enhanced by those polyphenol-rich foods that were identified in the NDNS RP analysis as particularly beneficial for lowering the odds of MetS.

#### 3.8.5.2 Low-carbohydrate, high-fat diets and the human metabolome

There are a number of emerging research areas that can shed further light on the debate surrounding the question of the healthiness of LCHF and EWG diets. A rapidly evolving area of research is the exploration of the human metabolome with the aim of gaining further insights into the metabolites arising from nutrients consumed and their associations with CM health and disease (McGarrah et al., 2018). A number of plasma metabolites have been identified that arise during lipid metabolism, such as fatty acids (Xie et al., 2012), that can be used as novel markers of CVD risk, such as branched-chain fatty acids (McGarrah et al., 2018), and in atherosclerosis, such as ceramides (Kordalewska and Makuszewski, 2015). Incorporating metabolic analysis into future LCHF trials will therefore offer the opportunity to better comprehend inter-individual differences in responses of varying CM biomarkers to the diet. Whilst the analysis of plasma metabolomic markers of CMD was beyond the scope of this PhD thesis, plans are underway to undertake this analysis in the near future.

Within the context of the food group-focused LCHF diets proposed in chapter 3.8.5.1 the metabolites arising from consumption of either meats or fermented dairy products and micronutrient-rich (polyphenol-rich) foods will be of special interest. Capel et al. (2019)

reported that the consumption of (low-fat) dairy products was associated with a less detrimental serum metabolomic profile in 61 individuals with MetS; this led to reduced inflammation and oxidative stress. In their analysis of data from 27,584 participants of the EPIC Potsdam PCS Wittenbecher et al. (2015) identified a number of lipid metabolites and amino acids that could provide links between red meat intake the the risk of T2DM. The sphingolipid ceramide has been associated with IR and increased inflammation and can derived from palmitic acids, which might have been synthesised through DNL (Mathews et al., 2017; Norris and Blesso, 2017). Replacing refined CHO with fruit and vegetables in 36 young adults reduced ceramide concentrations, potentially lowering CM risk in the participants (Mathews et al., 2017). Wang et al. (2017) found that following a Mediterranean diet pattern reduced plasma ceramide concentrations and mitigated CM risk. Investigating the effects of differently-composed LCHF diets might therefore elucidate whether specific LCHF dietary patterns are prefereable to maintain CM health.

#### 3.8.5.3 Low-carbohydrate, high-fat diets and the human microbiome

In recent years, the human microbiome has emerged as an important factor in diet and disease. There appear to be associations between the composition of the microbiome and the risk of presenting with CMD (Hansen et al., 2015; Yoshida et al., 2018). There is now strong evidence that the bacteria in the human gut produce substrates and metabolites that can be beneficial for or detrimental to CM health, such as trimethylamine-*N*-oxide (TMAO) (Yoshida et al., 2018). Trimethylamine-*N*-oxide is associated with increased risk of CMD (Janeiro et al., 2018). Its production seems to be impacted by consumption of red meats (Wang et al., 2019). Beneficial metabolites include short-chain fatty acids (SCFA), which are derived through the fermentation of dietary fibre in the gut (Hansen et al., 2015, Zhao et al., 2018). A number of other dietary factors have been recognised that impact the microbiome and potentially in consequence human health (Zhang et al., 2018; Zmora et al., 2018). The candidate is not aware of any studies that have investigated the impact of LCHF diets on the human microbiome and CMD factors. Future research on LCHF diets needs to bridge this gap in order to elucidate further mechanisms that contribute to the effect that LCHF diets have on surrogate markers of CM risk.

Of special interest in this context are also polyphenols, which would be an additional factor in designing the LCHF diets proposed in chapter 3.8.5.1. Polyphenols have been found to not only exert beneficial effects on blood pressure and other atheroslcerotic risk factors, but to also impact the microbiome by increasing the number of beneficial bacteria (Fraga et al., 2019). It would therefore be of special interest to examine not only whether LCHF diets

focused on either meat or fermented dairy products exert differential effects on the microbiome and detrimental gut-derived metabolites, but also whether carefully including polyphenol-rich foods would counteract the negative effects that meat consumption (TMAO-producing) could have.

#### 3.8.5.4 Low-carbohydrate, high-fat diets and the impact of genetic factors

Another aspect to generally consider is the impact of genetic factors on whether different dietary strategies are successful in maintaining CM health (Goni et al., 2016). Gardner et al (2018) recently investigated the impact of genotype on the success of a 12-month LC vs. a LF diet and found that genotype pattern did not influence weight loss. However, other genotypes, such as the FGF21 genotype, appear to be a determining factor in the loss of abdominal AT and change in body composition (Heianza et al., 2016). The researchers found that those carrying specific alleles of this genotype lost marginally less abdominal AT and significantly less total FA when following a LCHF diet. Those carrying the same allele and followed a HCLF diet had significantly more success in reduction of these AT depots.

Of special interest in the context of diet-gene interactions would be to determine whether LCHF study participants present with the the single nucleotide polymorphisms (SNP) ApoE4 genotype as this interacts with the amount and type of dietary fat and responds with increased CMD risk (Minihane et al., 2007). In ApoE4 carriers LDL-C concentrations tend to be higher which is mechanistically caused by alterations in the protein structure leading to increased lipid-binding abilities to VLDL particles. Consequently, these remain longer in the circulation with potential atherogenic effects (Phillips, 2014). However, other SNPs impacting diet-gene interaction within the context of dietary fatty acid consumption have also been reported (Dimitriou and Dedoussis, 2012; Fenwick et al., 2019; Konstantidinou et al., 2014).

With rising awareness that personalised nutrition might hold the key to improving the lives of individuals it would be advantageous for a future LCHF vs. EWG dietary intervention to take into account genotype when assessing the effect of the diets on surrogate markers of CM risk. Of special interest would be to investigate how much genotype would influence the response to differently composed LCHF diets as outlined in chapter 3.8.5.1. Would response to diet be mainly be influenced by their fatty acid content and composition in specific genotypes, or would the food matrix and high amounts of polyphenyl-rich foods have a confounding role to play?

### 3.9 Study strengths and limitation

#### 3.9.1 Study design

The CALIBER study is to the candidate's knowledge the first study designed to compare a low-carbohydrate, high-fat diet to a diet following the UK dietary guidelines in terms of adherence and the diets' impact on surrogate biomarkers and other factors associated with increased CM risk.

The short study period of eight weeks made it impossible to contribute to the body of evidence on medium- and long-term LCHF diets or the UK dietary guidelines within the context of dietary intake and CM risk. However, this is not the first LCHF study of this or shorter duration (Bradley et al., 2009; Sharman et al., 2004) and the timeframe is sufficient to investigate changes in markers that have a fairly short half-life and stabilise within a short period of time. Mensink (2016) noted that after 13 days a steady-state for lipids and lipoproteins would be reached. Other markers associated with food intake exert both acute and chronic responses. These include insulin and the hepatokine FGF21 (Hill et al., 2018; Schenk et al., 2008). In addition, adherence to dietary interventions tends to decline over time and in some studies participants reported difficulties with adherence at six months (Stanton et al., 2017: Gjuladin-Hellon et al., 2019). The eight-week period therefore had a high likelihood of high adherence levels.

Many previous studies comparing LCHF and higher-carbohydrate diets instructed their participants to follow the LCHF diet ad libitum but introduced an energy-deficit for the HC arm (Brehm et al., 2010; Bueno et al., 2013; Foster et al., 2003; McClernon et al., 2007; Vetter et al., 2010), thus introducing bias as the energy-restriction rather than the diet composition might have the desired effects of improvement of CM risk factors. Instructing both groups to follow the allocated diets ad libitum like in the present study reduced this bias.

#### 3.9.2 Recruitment

Despite best efforts, it was not possible to meet the initial recruitment target of 10 participants per group and only 50% of the target was met. This is a common problem in the recruitment for health-related and clinical trials (Carlisle et al., 2015; Walters et al., 2017). Nonetheless, due the nature of the intervention as a pilot feasibility study despite under-recruitment valuable information was gained inform the design of a main trial (Whitehead et al., 2016) .If the pilot were to be rolled out as a larger RCT sample size would be determined based on a two-sided significance level of $p<.05$ with a power of 80%. The effect size would be decided once a final decision had been made which surrogate marker in

particular to use as primary outcome measure. In addition, an attrition rate of around 20% would be taken into account. Nutrition intervention trials and health-interventions tend to suffer from high attrition rates and one of the present study's definite strengths was therefore that it achieved a completion rate of 100%, which is very often not the case (Walters et al., 2017).

Further studies leading on from the CALIBER study would focus on sdLDL-C as primary outcome as the findings for this CM surrogate marker were significant and very distinguishable between the two intervention group. A power calculation was undertaken using an online sample size calculator (https://clincalc.com/stats/samplesize.aspx). Based on the endpoint concentrations obtained for sdLDL-C (.93 mmol (SD .31) for the LCHF group and 1.00 mmol (SD .41) for the EWG group) with a power of .8, α (type I error) set at .05 and β (type II error) set at .2 (common values in sample size calculations (Noordzij et al., 2011) the total number of participants required in a subsequent trial would be n=616. Taking into consideration an attrition rate of 20% total number of participants required would be n=616*1.2=740. This would mean that for each diet group 370 participants would be required.

Although interest in the study seemed to have been initially high, as the number of accesses to the BOS screening questionnaire in response to seeing information on the study advertised somewhere, demonstrated (n = 540), the vast majority of people decided to not proceed further. This might have been due to study eligibility or subsequent hesitation to commit to the trial. However, a confounding factor impacting the decision not to proceed further might have been that LJMU REC stipulated as a condition for the ethical approval of the study for the PIS and informed consent form to be posted on the first page of the survey, before candidates would even attempt to answer the first question of the screening questionnaire. This meant that a candidate had to read more than 3,000 words before proceeding to the actual screening questionnaire. On average, a college-educated individual reads and comprehends about 250 words per minute (Taylor, 1965, see also Obar and Oeldorf-Hirsch, 2018). This means that it would have taken at least 12 minutes before a first attempt to complete the screener. This might have led to a high number of respondents not proceeding further. In addition to the large amount of information posted before the questionnaire the very formal and academic language of the PIS and ICF might also have played a role in the limited number of candidates proceeding to the first question of the screener. The 2011 Skills for Life survey found that 43% of adults had literacy skills below a level that would allow them to compare products and services to make best buy decisions, equivalent to a lower

GCSE grade (Department for Business, Innovation and Skills, 2011). The National Work Group on Literacy and Health recommends that literature aimed at patients should be target to the reading age of 11-12 year olds (Fitzsimmons et al., 2010) . Bearing this in mind there might have been a considerable number of potential candidates, who simply found the readability of the first page of the online survey to be low and therefore did not proceed further. Readability of health-information materials is a common problem Morony et al. (2015). To increase potential recruitment rates in future studies the candidate would therefore consider using methods of quantitative readability, such as Flesch-Kincaid (F-K), Simple Measure of Gobbledygook (SMOG) and Felsch Reading Ease (FRE) formulae (Cheng and Dunn, 2015; Fitzsimmon et al., 2010) to assess suitability of recruitment and study information material prior to recruitment. Not taking into account readability of the recruitment material might have also meant that potential participants from lower socio-economic groups, which tend to have lower levels of literacy Gilbert et al. (2018), are more likely to have lower levels of health literacy and consequently display higher CMD risk (Magnani et al., 2018) , were not reached. This would mean a missed opportunity of potentially decreasing inequalities in health. Social media have been deemed a valid and useful tool to attract initial interest in health-related studies. However, they do not always lead to successful recruitment and retention (Alley et al., 2016; Frandsen et al., 2016; Topolovev-Vranic and Natarajan, 2016) . Depending on the complexity of the study, a more personalised follow-up once initial contact has been made to establish rapport with the candidate as soon as possible should be considered (Brueton et al., 2014; Huang et al., 2018). Likewise, interventions that are more complex need to consider the level of literacy and health literacy of their target group. The number of people that were approached face-to-face but declined to complete the screening questionnaire was not recorded. The number of people reached via university email is also unknown. To avoid this limitation in future a separate log would help to record the number of times an individual was approached face-to-face for study recruitment and note whether they agreed to screening or not. Likewise when enlisting the help of university administration staff to send recruitment emails to a general university mailing list, in future it would help completeness of the data to enquire about the total number of contacts on these mailing lists. It is also unclear where those accessing the online questionnaire had obtained the link from (posters, social media, word-of-mouth). In future the candidate would therefore include a question at the very beginning "How did you find out about this study" with several options for the responder to choose from ('Social media', 'Poster/flyer', 'Word-of-mouth', 'Other').

To translate the experiences with the recruitment to more successful recruitment rates in a future full RCT the approach and protocol developed by Rooshenas et al. (2019) will be applied when possible at the refinement stage. Their QuinteT Recruitment Intervention (QRI) is to be integrated into the RCT protocol to aid recruitment rates and retention. Whilst they divide the intervention into two stages during the recruitment process of the ongoing RCT, the experiences from CALIBER can serve as a contribution to the second stage of a future CALIBER intervention. The experiences gained can already aid improvement of recruitment strategies. Admittedly, the application of qualitative data that is to be collected for stage 1 for any ongoing trial according to QRI is somewhat limited as only the final interview from the CALIBER pilot study can be used to inform of any issues regarding participation in the trial. These can be used in accordance with the QRI protocol to find practical solutions for improving recruitment and retention.

### 3.9.3 Dietary intake, adherence and food cravings

Self-reported assessment methods of dietary intake, such as the FFQ and the FD used in the present study, have a number of limitations. Overall, self-reported methods are known to be prone to reporting bias caused by the desirability or socially unacceptability of some food items (Desroches et al., 2013; Subar et al., 2015). For the FFQs it might have been more difficult to remember accurately regular consumption of food items in the correct frequency and amount (Desroches et al., 2013; Sham et al., 2014), whereas the completion the four-day food diaries was more immediate as participants were instructed to record foods and drinks as they consumed them. Nonetheless, being conscious of taking part in a dietary study participants might have either changed their consumption habits subconsciously on those days due to desirability/undesirability of certain items or due to the burden of having to record everything they ate (Subar et al., 2015).The FFQ was analysed using the appropriate software (FETA) with pre-specified food portion sizes. These might differ from individual participants' portion sizes for certain food and drink items. Partridge and colleagues (2018) found significant discrepancies between foods assessed by volume (e.g. mug, spoon etc) and dietary assessment databases standardised weights, more so for dairy and high-protein foods than for fruit and vegetables. Researcher bias when processing the FDs might have also resulted in discrepancies between actual and assumed food portion sizes. However, only one of the differences in self-reported intake was significant. One advantage of the study was the consistency of data entry. All FDs were processed in Dietplan 7 by the candidate who ensured to use the same codes for items consumed on a regular basis. Furthermore, all FDs were collected and analysed together after the study had ended, ensuring consistency (e.g. food codes, portion sizes) when entering the data. The candidate also went through FDs

briefly with the participants during laboratory appointments to clarify any obvious and major issues and to get an idea of participants' food habits and portion sizes. Carbohydrate intake in the EWG group was 5.4% and 7.9% below EWG recommendations at interim point ad endpoint. Even increasing researcher-estimated or standardised food portion sizes would have been unlikely to bring mean CHO (%TE) intake in the EWG group to or even above the 50%TE threshold.

Despite dietary records supporting the assumption that the LCHF group adhered to the limits of 50 g/d of CHO intake, plasma hydroxybutyrate levels seem to contradict this with no participants at endpoint managing to achieve the 0.5mmol/L ketone bodies threshold. Although the manufacturer's technical specification stated that EDTA plasma was suitable for analysis, Custer et al. (1983) found that anticoagulant interfered with the determination of 3-Hydroxybutyrate by as much as 37%. However, even if an additional 37% were to be added to the mean values of 3-Hydroxybutyrate obtained in this study, volumes would remain below the 0.5 mmol/L threshold to indicate a state of nutritional ketosis. In future, an additional and more immediate method of validating adherence should therefore be considered. Participants could be given single-use dipsticks to self-test their urine on a daily basis for the ketone bodies acetoacetate to confirm presence of nutritional ketosis and keep a log of the results. This way the researcher can get a better impression of adherence already at interim point (and where necessary provide additional dietary consultations to the non-adhering participants) and check whether nutritional ketosis was achieved at any point during the study (Urbain et al., 2017). In addition, this could serve as a method of motivating the participants in the LCHF as they can see easily whether their CHO intake is sufficiently low to produce ketone bodies (Hallberg et al., 2018).

Not all participants in the EWG group adhered to the instructions of increasing CHO intake to ≥50%TE. Therefore, CHO consumption could only be classed as moderate. Perhaps it is easier for individuals to strictly limit consumption of food items and food groups rather than to attempt what might be perceived as small dietary changes. Although both groups received guidelines and meal plans (plus recipes) to support them with their diets, a more targeted approach is required in the future. Rather than focus on the diet overall, focus should be placed on food items that can be the key to meeting UK dietary recommendations. Using decision trees and utilising data from the NDNS RP 2008-2012 Giabbanelli and Adams (2016) identified 11 foods that would predict whether dietary recommendations for fruit and vegetables were achieved (83% accuracy) and 32 foods for fat (plus age) to achieve recommendations for fats (72% accuracy).

Another point to take into consideration in the current analysis was that 80% of the study sample was male, with one female participants each in the LCHF and the EWG group. There is evidence that gender impacts food cravings (Hallam et al., 2016) with men craving more savoury than sweet foods and also different types of sweets. Hormonal fluctuations mean that those gender differences are more pronounced with pre-menopausal women (McVay et al., 2012) . Only one of the women in the present sample was pre-menopausal. Furthermore, the FCI-UK assesses cravings over a whole month so that fluctuations of food cravings over the menstrual cycle should be evened out. The two FCI-UK questionnaires were completed with four weeks between them so that hormone levels should have been similar between visits.

### 3.9.4 Surrogate biomarkers of cardiometabolic risk

This study was the first study comparing the effects of a prescribed ketogenic diet with a prescribed EWG diet on FGF21 concentrations to gain further insights into the complex nature of dietary intake and CMD. A wide range of markers was measured adding to the strength of the study, although the sample size was small. The study also took into account that there were inter-individual differences in responses to the two diets that deserve further attention in future. Furthermore, the composition of the saturated fatty acid and CHO profiles and the intake of food groups associated with them was investigated. Although sdLDL-C cholesterol and apoB were measured in acknowledgement of the discordance between LDL-C concentrations and other associated atherogenic lipids and lipoproteins, it would have been even more informative to assess sdLDL particle numbers. This would have allowed for even more accurate profiling of CM risk. However, this was not possible due to financial constraints within the remits of this PhD study. The %CV for the within run for apoB and fasting blood glucose was not obtained. However, the kits used have been validated and should therefore provide accurate measures. In addition, neither of these markers were statistically significant and they were therefore not considered for inferences on the impacts of the two diets. All blood samples were analysed within a maximum of six months from when they were obtained, so that the integrity of the biomarkers will have been retained.

### 3.9.5 Body composition

Although validated, BIA is not as accurate as the gold standard methods, DXA and MRI. The algorithms used by the equipment to estimate VAT have improved but they still tend to over-or underestimate VAT (Day et al., 2018). This is more the case in obese populations, whereas the present sample was drawn from normal-weight and overweight individuals. Furthermore, the participants were asked to drink a large glass of water prior to their

assessments to aid hydration. The limitations of BIA were less severe within the context of the present study as three measurements were taken at three time points and the focus was on changes in adiposity and not cross-sectional data.

It has been stated in the past that the weight loss experienced on a LCHF diet is due to water weight loss (Bortz et al., 1967; Yang and Italie, 1976). Brehm et al. (2009) argued that these studies were of very short duration (≤2 weeks) and that the effect of diuresis was temporary. The first assessments in the present study were taken after four weeks on a LCHF diet and in accordance with Brehm et al. (2009), it can be assumed that the weight loss went beyond diuresis. Measuring FM and VAT therefore added strength to this study as the source of the weight loss was quantified.

### 3.9.6 Physical activity assessment

As one participant in the final analysis sample did not provide at least one valid weekend day at endpoint due to non-compliance with the seven-days wear time of the ActiGraph GT9X accelerometers, data from an unweighted week was used for analysis and all days, regardless of being weekend days or week days, were treated equally. Physical activity behaviour tends to be different at weekends compared to weekdays (Drenowatz et al., 2016). Therefore it is common practice to include at least one weekend day in the analysis and to calculate weighted averages of weekday and weekend physical activity (Marshall et al., 2015). However, the present study undertook repeated measurements over three time points to assess changes in MVPA time, and as the sample size was small, it was deemed acceptable to proceed with data from an unweighted week. Previous studies have justified using just one valid day to be included in the analysis (Tudor-Locke et al., 2009; Schuna et al., 2013).

## Chapter 4 - Overall summary, conclusions and outlook

The aim of this thesis was to examine potential links between intake of dietary CHO and SFA and surrogate markers of CM risk. For the first study (chapter 2) secondary analysis of the NDNS RP 2008-2014 was undertaken, which found that those with lower CHO or higher SFA intake had decreased odds of presenting with MetS. Based on these findings, for the second study (chapter 3) a pilot feasibility trial was designed in which participants were randomised to one of two groups that reflected these opposite intakes, a LCHF diet and a diet following the EWG. To some degree the second study confirmed the findings of the first but it also exposed the magnitude (within the limitations of the sample size) of inter-individual variations in responses to the diets. Further analysis of the NDNS RP 2008-2014 sample used in this PhD thesis could be undertaken to confirm whether these inter-individual responses also exist in the general UK population.

The findings from both studies highlight the need to view dietary intake within the context of the food matrix, rather than taking a reductionist approach. Foods are more than the sum of their parts and by simply looking at macronutrient intake, the synergistic effects of the nutrients contained within the matrix are easily missed. In future, dietary intervention studies should this into account. This will strengthen the evidence base that informs and shapes national dietary guidelines.

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## Chapter 6 – Appendices

**From:** Williams, Mandy <A.F.Williams@ljmu.ac.uk>
**Sent:** 03 January 2017 13:41
**To:** Harrison, Tanja <T.Harrison@2015.ljmu.ac.uk>
**Cc:** Davies, Ian <I.G.Davies@ljmu.ac.uk>; Lane, Katie <K.E.Lane@ljmu.ac.uk>; Boddy, Lynne <L.M.Boddy@ljmu.ac.uk>; researchethics <researchethics@livjm.ac.uk>
**Subject:** Approved - Harrison (with attached approved application)

Dear Tanja

With reference to your application for Ethical Approval:

**16/ELS/029 - Tanja Harrison, PGR - CALIBER (Carbohydrates, lipids and biomarkers of traditional and emerging cardiometabolic risk factors): A pilot study in normal-weight and overweight adult males (Ian Davies/Katie Lane/Lynne Boddy)**
The University Research Ethics Committee (UREC) has considered the above application. I am pleased to inform you that ethical approval has been granted and the study can now commence.

Approval is given on the understanding that:

- any adverse reactions/events which take place during the course of the project are reported to the Committee immediately;
- any unforeseen ethical issues arising during the course of the project will be reported to the Committee immediately;
- the LJMU logo is used for all documentation relating to participant recruitment and participation e.g. poster, information sheets, consent forms, questionnaires. The LJMU logo can be accessed at http://www.ljmu.ac.uk/corporatecommunications/60486.htm

Where any substantive amendments are proposed to the protocol or study procedures further ethical approval must be sought.

Applicants should note that where relevant appropriate gatekeeper / management permission must be obtained prior to the study commencing at the study site concerned.

For details on how to report adverse events or request ethical approval of major amendments please refer to the information provided at http://www.ljmu.ac.uk/RGSO/93205.htm

Please note that ethical approval is given for a period of five years from the date granted and therefore the expiry date for this project will be January 2022. An application for extension of approval must be submitted if the project continues after this date.

LIVERPOOL JOHN MOORES UNIVERSITY

**Mandy Williams, Research Support Officer**

**(Research Ethics and Governance)**
**Research and Innovation Services**
Kingsway House, Hatton Garden, Liverpool L3 2AJ
t: 01519046467 e: a.f.williams@ljmu.ac.uk

**From:** Williams, Mandy <A.F.Williams@ljmu.ac.uk>
**Sent:** 12 July 2017 10:35
**To:** Harrison, Tanja <T.Harrison@2015.ljmu.ac.uk>
**Cc:** Research Ethics Proportionate Review <EthicsPR@ljmu.ac.uk>
**Subject:** Major Amendment Approval - Harrison (16/ELS/029)

Dear Tanja

Further to the above applications for major amendments which you recently submitted for consideration by the University's Research Ethics Committee. Please accept this email as formal confirmation that REC agreed to approve this application by Chairs action.

**Mandy Williams, Research Support Officer**

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29th November 2016

Dear Dr Harriss

**Re: Dietetic opinion – ethics application.**

I am writing to provide an independent dietetic opinion regarding the protocol submitted by Tanja Harrison and Dr Ian Davies for their proposed trial involving a very low carbohydrate diet.

Whilst I understand the committee's concerns regarding the potential side effects of both the regime itself and its knock on effect on other nutrients, namely fibre, I have experience of recommending such dietary regimes both in clinical practice and for research purposes and feel that the real risk in the proposed population group will be low and can be appropriately managed through procedures already planned by the research team and reiterated in my response below. In formulating these options of the proposed study the following key facts are of note:

1. This is a healthy population with those at higher risk of suffering adverse events or with existing medical conditions excluded from participating.
2. This is a relatively short study (compared to others in the literature including our own 6 month trial) with each level of carbohydrate prescribed for a maximum of 4 weeks
3. This is a free-living sample consuming an ad libitum diet meaning that over consumption of carbohydrate is more likely than under consumption and participants will be able to eat to appetite from other foods outside of their carbohydrate restrictions
4. The carbohydrate limits set out in the protocol represent only available carbohydrate meaning that total carbohydrate intakes will be higher than this, due to the inclusion of high fibre, less digestible sources, which will again make the diet more tolerable, palatable and less likely to exert side effects.

With respect to your specific concerns:

1. Negative impact on cognition

Our experience of low carbohydrate diets has shown us that people respond very differently to these and therefore the absolute level of carbohydrate is often a poor marker for the effects of the diet, both anticipated and unexpected. Some people do certainly feel some dizziness in the early stages of a low carbohydrate diet, when they are adapting to the change in macronutrient balance and/ or when they achieve a state of ketosis, but this is usually fairly short-lived and in fact we ourselves ran a study combining low carbohydrate diets with increased exercise which helped to dispel some of the myths linked to these types of regimes. Of course all real or perceived symptoms experienced by participants should be taken seriously and managed accordingly but having a robust reporting procedure in place for any such adverse events, e.g. an out of hours contact and the establishment of a good rapport between researchers and participants will minimise any associated risk. Informing participants upfront of the likely side effects of the diet, encouraging them to start the diet on a less busy day/ week and engaging them in meal planning/ providing shopping lists to ensure they have suitable foods available will all help to minimise risk.

2. The need for GP involvement/ authorisation

In our experience the majority of GPs are not familiar with these dietary regimens and therefore may not be best placed to advise on the suitability for their patients. Assuming the participants meet the inclusion criteria set by the research team and consent to the study, including understanding and accepting their responsibility to report any adverse events they should be safe to follow the diets proposed. We would recommend that an additional exclusion criteria of 'current or previous renal impairment' be added to screen out any participants with impaired renal function for whom a higher protein diet (as often results from lowering carbohydrate) may pose a slightly higher risk. Renal impairment would be unlikely within the proposed study sample.

3. Strategies to maintain fibre intakes

Whilst low carbohydrate diets have the potential to be low in fibre, in our experience the tailored advice given during a research study, including the focus on high fibre / low carb sources means people are likely to maintain, if not improve, their fibre intake relative to baseline. In my opinion the aim should always be to maintain fibre intakes relative to each individual's baseline rather than trying to bring all participants up to a higher level (e.g. the UK recommendation of 30g) as this will create additional confounding in the study design as well as potentially causing gastrointestinal side effects of its own. As such the plans outlined by the research team to highlight and encourage higher fibre, lower carbohydrate foods and ingredients should be more than capable of maintaining fibre levels over this relatively short period of time. The research team can easily monitor compliance with this higher fibre advice at scheduled study visits through the use of a simple checklist of the recommended foods as well as a structured adverse events interview including both open questions and closed questions asking about all potential side effects such as constipation and fatigue. Fibre supplements are not generally recommended as these are associated with side effects of their own. Increasing fibre intakes gradually via food alongside increase in fluid is usually better tolerated.

4. Consequences of a low fibre diet

Some people may experience a change in bowel movements and in particular increased constipation as a result of reducing their carbohydrate intake and subsequently reducing their fibre and increasing their protein. However, as discussed above, it is likely that the participants in this study will in fact maintain or even increase their fibre intakes and therefore the risks of constipation are minimised. These can be further reduced by encouraging participants to increase their fluid intakes and to take gentle exercise (again avoiding any dramatic changes in activity levels which may confound the study findings). Once again if participants are informed of these potential issues before they start the study and are encouraged to purchase the higher fibre foods listed both the chance of them being effected and the impact of any adverse event will be reduced.

This is an interesting and timely study and I look forward to hearing how it proceeds. If you require any further information please do not hesitate to contact me.

Yours sincerely

Dr Kathryn Hart

# CALIBER – Screening questionnaire

*Please circle answers*

Date of survey completion

Are you male or female?

Male Female

Are you between 19 and 64 years of age?

Yes No

What is your exact age?

*Women only*

Would you describe yourself as

Pre-menopausal ☐

Peri-menopausal (periods starting to be more irregular in recent months/years and as you have aged) ☐

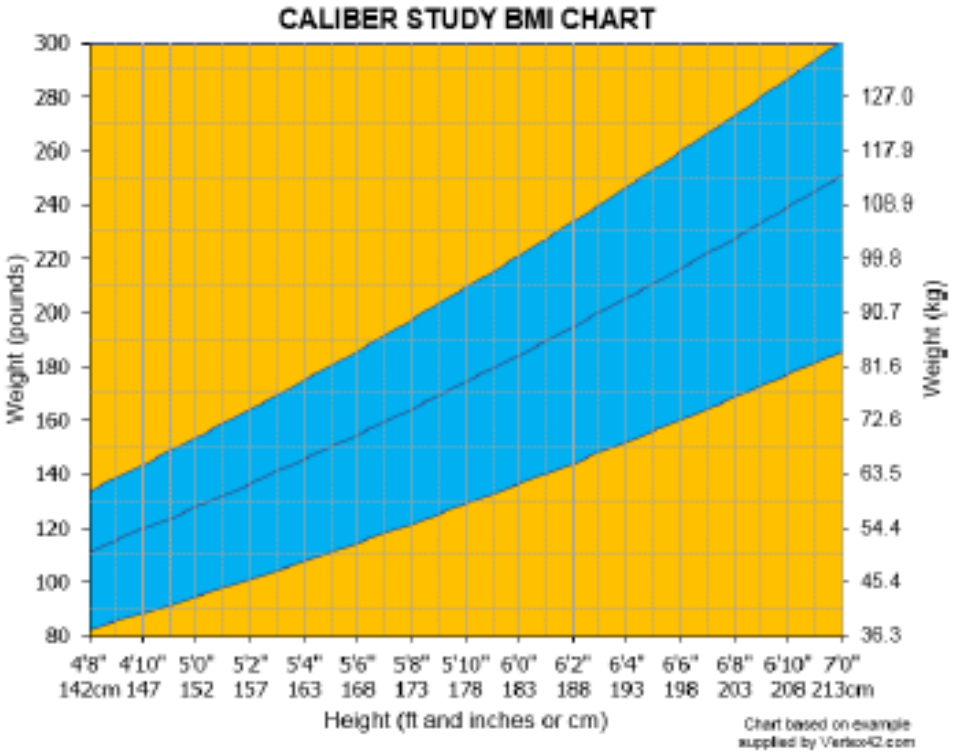
Post-menopausal (last period more than 12 months ago)? ☐

What is your height (in m or ft. & inches)?

What is your weight (in kg or stone)?

Below you can see a chart to work out your BMI. Based on your weight and height is your BMI in the blue range?

Yes No




Do you know your waist circumference?

Yes No

If yes can you please enter your waist circumference in the box below and indicate whether this is in cm or inches?

Would you describe your ethnicity as White Caucasian?
Yes No

Do you drink alcohol?
Yes No

If yes Do you drink more that the recommended allowance of 14 units per week (=6 pints of lager or cider; or 6 175ml glasses of wine; or 14 25ml glasses of spirit)
Yes No

Do you smoke?
Yes No

Have you ever smoked?
Yes No

If yes, did you stop smoking less than 12 months ago?
Yes No

Are you a vegan or vegetarian?
Yes No

Have you been diagnosed by a medical professional with any food allergies or intolerances? E.g. lactose intolerance, gluten intolerance, food allergies
Yes No

Do you take any dietary supplements (such as vitamins/minerals, omega-3 oils)?
Yes No

Have you ever been diagnosed with an eating disorder, such as anorexia nervosa, bulimia, binge eating disorder (BED) or an eating disorder otherwise not specified (EDONS)

Yes No

Have you ever been diagnosed with impaired kidney function

Yes No

Have you ever been diagnosed with any cardiometabolic diseases, such as heart disease or type 2 diabetes?

Yes No

Do you take any medication against high cholesterol, high blood pressure or high blood sugar?

Yes No

Would you be interested in being contacted for further screening to confirm some details and to provide a small fasted blood sample via finger prick?

Yes No

If yes "Can you please provide us with your name, email address and telephone number and indicate which contact method you prefer?"

*Thank you for taking the time to participate in this survey and your interest in our study. It is very much appreciated.*

For office use only – Please add participants ID once allocated after finger prick and consent.

Participant Information Sheet

CALIBER (Carbohydrates, lipids and biomarkers of traditional and emerging cardiometabolic risk factors) Phase 1: A pilot study in normal-weight and overweight adults.

(Researcher: Tanja Harrison, Faculty of Education, Health and Community, Liverpool John Moores University)

You are being invited to take part in a research study. Before you decide it is important that you understand why the research is being done and what it involves. Please take time to read the following information. Ask us if there is anything that is not clear or if you would like more information. Take time to decide if you want to take part or not.

*Please be aware that you will be excluded from the study if you under the age of 19 or over the age of 64, non-White Caucasian, smoker, vegan/vegetarian, take dietary supplements, have any known food allergies or intolerances, consume alcohol above the weekly UK recommendations, suffer from an eating disorder, suffer from current or previous renal impairment, have a history of cardiometabolic diseases, or take lipid, blood pressure or blood glucose-lowering medication, or if your BMI is below 18.5 or over 30kg/m$^2$. Final eligibility will be based on obtaining a score between 4 and 11 in the following points system: fasting blood sugar concentration >5.5mmol/L = 3 points; waist >102cm or 40 inches for males; waist >88cm or 34 inches for females= 2 points; waist >94 cm or 37 inches for males; waist >80 cm or 31 inches for females = 1 point; systolic blood pressure >130mmHg = 1 point; diastolic blood pressure >85mmHg = 1 point; HDL cholesterol concentration <1.0mmol/L for males; HDL cholesterol concentration <1.3mmol/L for females = 2 points; serum triglyceride concentration >1.3mmol/L = 1 point. Dependent on the number of markers contributing to your score this might mean that you are at increased risk of pre-metabolic or metabolic syndrome and potentially at increased risk of developing cardiovascular disease. Should the following thresholds found to have been exceeded during the screening process you will be excluded from the study, and we would strongly advise you to see your GP: blood glucose ≥7mmol/L, triglycerides: ≥5.7mmo/L or systolic blood pressure ≥160mmHg or diastolic blood pressure ≥ 100mmHg.*

*Please note that the method used during the screening process for assessing your blood markers (via finger prick) is used within a research rather than a clinical environment (such as a hospital or a GP practice). Although the finger prick method used is scientifically accurate it might not be as precise as other methods used clinically and might differ from those obtained through your GP or a hospital setting. This means that our scoring system might deem you to be at increased risk of developing metabolic syndrome and cardiovascular disease when this is not the*

*case. However, should you be concerned about any of the findings obtained during the screening process we would advise you to seek further advice from your GP.*

What is the purpose of the study?

The purpose of this study is to investigate the effects of very low-carb, high-fat (VLCHF) or high-carb, moderate fat (HCMF) diets on traditional and novel potential risk markers for heart disease and type 2 diabetes in normal-weight and overweight adults aged 19-64 in a free-living situation. This will be in form of an 8-week pilot study. The study will take place at Liverpool John Moores University.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do you will be given this information sheet and asked to sign a consent form. You are still free to withdraw at any time and without giving a reason.

What will happen to me if I take part?

If you agree to take part, you will be:

Asked to provide some personal details (e.g. name and contact details) to take part in the study

Asked to complete a brief screening questionnaire which takes a maximum 10 minutes to complete. The questionnaire uses a screening out process for those participants that do not meet the inclusion criteria. The questionnaire is also available online.

The questions you will be asked include:

- Demographic and anthropometric questions
- Lifestyle questions (smoking and alcohol consumption)
- History of cardiometabolic diseases or taking medication against known cardiometabolic risk factors
- Whether you would be willing to take part in a further screening step, where you will be asked to provide a small fasted blood sample via finger prick

If you are eligible to take part after we have taken a small blood sample via finger prick we will require you to

- Adhere to an 8-week dietary intervention on an either very low-carb, high fat or a high-carb, moderate-fat. Please note that no meals or food will be provided during these phases. For each diet you will be provided with an information pack containing **recipes**, **example meal plans** and **shopping lists**, and lists of **permitted or recommended food** items. This information will also be made available online through a private Facebook Group.

On the very-low carbohydrate diet a minimum 30g and maximum 50g of energy will be derived from available carbohydrates. During this time your body will produce ketone bodies to provide it with energy.

- Be randomised into 1 of 2 groups; one which will follow a very low-carb, high-fat diet and one which will follow a high-carb, moderate-fat diet according to UK dietary recommendations as issued in the Eatwell Guide. You will be welcome to access any advice given to the other group (e.g. recipes, meal plans) after the study has finished.
- Access a range of **supporting materials** in a private Facebook group, such as **portion size** guides, **recipes** and **example meal plans**. However, should you not wish to join the group all materials will be provided to you as paper-based copies
- Agree for the person in your household who mainly does the food shopping and cooking to also have access to the private Facebook group and intervention materials (or the paper-based versions of these), such as portion size guides, recipes and example meal plans. No personal clinical test results will be shared with this person.
- To complete on **one occasion** (between 20 and 30 min completion time per task)
- a **field-based fitness test**. We will ask you to walk one mile as quickly as you can. During this test we will ask you to wear a heart rate monitor which involves wearing a band around your chest and a mobile phone in your pocket.
- a **food frequency questionnaire** assessing dietary intake over the past 12 months
- a recent **physical activity questionnaire** assessing physical activity over the past 4 weeks
- an **interview** using a semi-quantitative questionnaire about your **experiences** with the diet allocated to you at the end of the study
- To complete on **two occasions** (between 5 and 10 min completion time per task)
- a brief interview on your **dietary fibre** intake over the previous 4-week period
- a brief **interview** on any adverse effects and events that you might have experienced over the previous 4 weeks that might be connected to the intervention (VLCHF group only)
- To complete on **three occasions** (between 5 and 90 min completion time per task)
- a 4-day **food diary**

- a **food cravings** questionnaire
- a questionnaire assessing self-reported **cognition** over the past 4 weeks
- Come to the laboratories on IM Marsh campus, Liverpool John Moores University) to
    - Provide a fasted venous **blood sample** (about 8 teaspoons of blood)
    - Have your **body composition** measured via bioelectrical impedance
    - Let us take some basic **anthropometric** measures
    - Let you take your **blood pressure**
- We will also ask you to wear a **physical activity monitor** on your non-dominant wrist for 7 consecutive days on 3 occasions. The activity monitor is similar in size to a watch and can be used to tell the time. We would like you to wear the monitor from the moment you wake up in the morning until you go to bed at night. The monitor should only be removed for water based activities, e.g. swimming, showering, and when playing sports/engaging in activities where watches are prohibited. You will receive guidance on how to wear the monitor and a diary to record when the monitor is put on and removed each day.
- We will ask your permission to store your blood sample in LJMU freezers, only accessible to authorised personnel.
- We will use your stored blood sample in this research study to investigate the levels of inflammatory markers (genes) in your white blood cells. This will give us a further indication of your cardiometabolic health.
- If you give us permission to, any remaining blood sample will be stored in a secure freezer for future ethically approved research. If you do not want us to do this the remaining sample can be disposed at the end of the current testing.

Once the study is complete you will be debriefed on your role in the study and given a chance to ask any questions.

Over the course of the study you will receive **regular reminders** via text message from us regarding different aspects of the intervention, such as completion of questionnaires and diaries, taking of supplements, wearing of physical activity monitors and appointment reminders.

**Are there any risks / benefits involved?**

You will be asked to answer all questions of the questionnaire honestly. All information and samples obtained as part of the study will be made anonymous so the answers provided by you will be unidentifiable in any further publications. After completion of the intervention you will receive £50 in high street vouchers and a personalised dietary consultation with an academic registered nutritionist (worthy of £250 per hour and

validated by a registered dietitian) based on your results from the intervention. You will also be entered into a price draw on study completion to win an additional £200 worth of high street vouchers.

The wider benefits of the pilot study are that the results can inform further studies to develop of future interventions and public health strategies to prevent, reduce or ameliorate cardiometabolic risk in the UK population.

There are some adverse effects associated with very low-carbohydrate diets which are expected to last on average no longer than a week. These include flu-like symptoms (headaches), constipation, bad breath, muscle cramps and general weakness. Some people have reported slight dizziness and 'brain fog' during this period of adjustment. You will be provided with information on how to deal with these potential side effects in the best possible way. This includes consuming sufficient amounts of fluids and eating potassium-rich and magnesium-rich foods. You will also be provided with a multi-vitamin and mineral supplement to ameliorate any adverse effects. Other potential risks during this phase include mineral deficiencies, lack of vitamins and low fibre intake, which if occurring over prolonged periods of time has been associated with increased risk of heart disease, cancer and bowel diseases. To prevent these risks from occurring you will be provided with a multivitamin and mineral supplement over the course of the intervention and be given guidance on fibre-rich foods that can be consumed. It is important to note that any potential risks will only be temporary and will not affect anyone who consumes a healthy, well-planned and balanced diet containing high-quality foods in the long-term. You will be provided with information on how to best deal with these potential side effects and be given out of hour contact details should you need further advice during this period.

**Will my taking part in the study be kept confidential?**

Completion of this study is voluntary. Samples will be stored with all identifiable information (e.g. name) removed. They will be stored in locked freezers only accessible to authorised personnel. Only the research team will have access to any personal information provided. Any personal information collected during this study will be stored confidentially and securely on password protected LJMU computers, hard drives, LJMU cloud space or in locked filing cabinets. The information provided by you will be unidentifiable in any publications, such as papers, written reports and presentations.

**This study has received ethical approval from LJMU's Research Ethics Committee (16/ELS/029 approved 14 December 2016)**

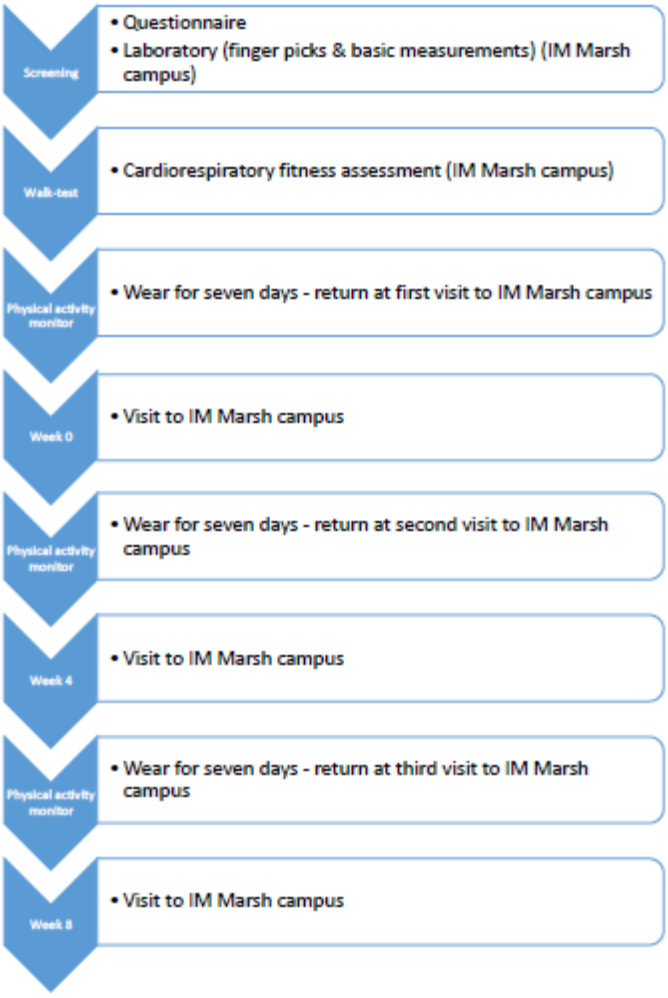
**Contact Details of Researchers**

If you have any queries regarding your participation or the study, please do not hesitate to contact

Tanja Harrison, email: t.harrison@2015.ljmu.ac.uk M 07970 858 594

Dr Ian Davies, email: i.g.davies@ljmu.ac.uk

**If you any concerns regarding your involvement in this research, please discuss these with the researcher in the first instance. If you wish to make a complaint,**


Screening
• Questionnaire
• Laboratory (finger picks & basic measurements) (IM Marsh campus)
Walk-test
• Cardiorespiratory fitness assessment (IM Marsh campus)
Physical activity monitor
• Wear for seven days - return at first visit to IM Marsh campus
Week 0
• Visit to IM Marsh campus
Physical activity monitor
• Wear for seven days - return at second visit to IM Marsh campus
Week 4
• Visit to IM Marsh campus
Physical activity monitor
• Wear for seven days - return at third visit to IM Marsh campus
Week 8
• Visit to IM Marsh campus

LIVERPOOL JOHN MOORES UNIVERSITY

LIVERPOOL HOPE UNIVERSITY

## Informed Consent Form

CALIBER (Carbohydrates, lipids and biomarkers of traditional and emerging cardiometabolic risk factors) Phase 1: A pilot study in normal-weight and overweight adults.

*Tanja Harrison, Faculty of Education, Health and Community*
*Contact details: t.harrison@2015.ljmu.ac.uk*
*Ethics Code: 16/ELS/029*

1. I confirm that I have read and understand the information provided for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. I do not meet any of the exclusion criteria outlined. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and that this will not affect my legal rights. ☐
3. I understand that any personal information collected during the study will be anonymised and remain confidential. ☐
4. I understand that I will be asked questions on demographics, anthropometrics, lifestyle, cooking and eating habits and history of cardiometabolic disease. ☐
5. I understand that I will need to adhere to an 8-week dietary intervention on an either very low-carb, high-fat (VLCHF) or high-carb, moderate fat (HCMF) (following the UK dietary recommendations) diet. On the very-low-carb, high-fat diet a minimum of 30g and a maximum of 50g per day of energy will be derived from carbohydrates. No meals will be provided to me during the intervention. ☐
6. I understand that there might be adverse events associated with the VLCHF diet of the intervention. I will inform the researcher of any adverse events that might occur to me whilst I am part of the study. ☐
7. I understand that should I be allocated to the VLCHF diet I will be provided with a multi-vitamin and mineral supplement, which I will need to take on a daily basis. ☐
8. I understand that I will be randomised into 1 of 2 groups, one consuming a VLCHF diet, the other a HCMF diet. I will be given access to any advice given to the other groups (e.g. recipes, meal plans) after the study has finished. ☐
9. I understand that I can access a range of supporting materials in a private Facebook group, such as portion size guides, recipes and example meal plans. If I do not wish to join the group paper copies of the materials will be provided to me. ☐
10. I understand that I will be required to complete a field-based cardiorespiratory fitness test (approx. 20-30 min completion time). ☐

11. I understand that I will be required to complete one food frequency questionnaire assessing dietary intake over the past 12 months (approx. 20 min completion time). ☐

12. I understand that I will be required to complete one recent physical activity questionnaire assessing physical activity over the past 4 weeks (approx.15 min completion time). ☐

13. I understand that I will be required to wear a wrist-worn physical activity monitor on 3 occasions (7 days for at least 10 hours per day per occasion). ☐

14. I understand that I will be required to complete a 4-day food diary on 3 occasions (approx. 20 min completion time per day). ☐

15. I understand that I will be required to complete a food cravings questionnaire on 3 occasions (approx. 5 min completion time per occasion). ☐

16. I understand that I will be required to complete a questionnaire assessing my self-reported cognition over the previous 4-week period on 3 occasions (approx. 5 min completion time per occasion). ☐

17. I understand that I will need to do an interview on my final visit to your laboratory on my experience with the me allocated diet over the course of the intervention using a semi-structured questionnaire. ☐

18. I understand that I will be required to come to the labs on IM Marsh campus, Liverpool John Moores University on 3 different occasions (up to 1.5 hours duration per visit) to ☐
    - Provide a fasted venous blood sample (about 8 teaspoons of blood)
    - Have my body composition measured via bioelectrical impedance
    - Let you take some basic anthropometric measures
    - Let you do a brief interview on my dietary fibre intake over the previous 4-week period (during my second and during my last appointment only)
    - Let you do a brief interview on any adverse effects and events that you might have experienced over the previous 4 weeks that might be connected to the intervention (VLCHF group only) (during my second and during my last appointment only)

19. I understand that I will receive regular text message reminders over the course of the intervention regarding different aspects of the intervention, such as completion of questionnaires and diaries, taking of supplements, wearing of accelerometers and appointment reminders. ☐

20. I agree for the person in my household who mainly does the food shopping and cooking to also have access to intervention materials (or paper-based versions of these), such as portion size guides, recipes and meal plans. I understand that no personal test results will be shared with this person. ☐

**Human Tissue Act (2004) informed consent**

21. I consent to my blood sample(s) being stored at LJMU, accessed only by authorised personnel. ☐

22. I consent to my blood sample(s) being used for the research purposes outlined in the Participant Information Sheet. ☐

23. I consent to my blood sample(s) being used in further ethically approved research, outside the scope of the current project. ☐

24. I give the consent for my blood sample(s) to be used for future DNA analysis or other genetic testing as part of a different ethically approved study. ☐

25. I agree to take part in the above study. ☐

| | | |
|---|---|---|
| Name of Participant | Date | Signature |
| Name of Researcher | Date | Signature |
| Name of Person taking consent *(if different from researcher)* | Date | Signature |

***Note to researcher:** When completed one copy to be retained by the participant. A second copy to be delivered to the HTA Coordinator, once the sample has been entered into the Pro-Cure database and the printed sample label affixed to the top right corner of this form and associated sample tube. Please retain a photocopy for your records in a locked filing cabinet.*

*For office use only. Participant number*

Gatekeeper Information sheet

**CALIBER (Carbohydrates, lipids and biomarkers of traditional and emerging cardiometabolic risk factors) Phase 1: A pilot study in normal-weight and overweight adults**

***Tanja Harrison, Faculty of Education, Health and Community***
***Contact details:*** *t.harrison@2015.ljmu.ac.uk*

**1. What is the reason for this letter?**

We would like to gain access to people who attend your organisation to invite them to take part in a research study investigating the impact of dietary carbohydrates and fats at different ratios on cardiometabolic risk markers.
Before you decide, it is important that you understand the purpose of the research and what it involves. Therefore, please take time to read the following information and decide if you want to accept or not. If there is anything unclear or you would like to obtain more information, please do not hesitate to ask us.

**2. What is the purpose of the study/rationale for the project?**

The purpose of the study is to investigate the effects of a very low-carb, high fat diet or a high-carb, moderate-fat (as recommended in UK dietary guidelines) diet on traditional and novel potential cardiometabolic risk markers in normal-weight and overweight males aged 19-64 in a free-living situation. This will be in form of an 8-week pilot study. The study will take place at Liverpool John Moores University.

**3. What we are asking you to do?**

If you agree to take part:

- We would ask for you to share information on the research via your online mailing lists, through people that attend your organisation or via displaying recruitment flyers for the study at your premises – this will require a small investment of your time.
- Potential participants in the study will be asked to initially complete an online screening questionnaire to determine their preliminary suitability for the study. Completion of this questionnaire is completely voluntary. At the end participants

- Asked to sign two disclaimers; one to take part in a cardio-respiratory fitness test suitable for a sedentary population, and another one to take part in an 8-week very-low carbohydrate, high-fat (~~ketogenic~~) diet
- Asked to provide some contact details
- Asked to complete a 20-30 minutes fitness test suitable for a sedentary population (1-mile walk test)
- Asked to complete a Recent Physical Activity Questionnaire and a Food Frequency Questionnaire on one occasion
- Asked to complete questionnaires on 3 separate occasions about food intake (4-day food diaries), cognition and food cravings
- Asked to complete a questionnaire on fibre intake and any adverse events that occurred (very low –carb group only) over the previous 4-week period on two separate occasions
- Asked to complete a brief interview using a semi-structured questionnaire on their experience with the diet on one occasion
- Asked to wear an accelerometer for 7 days on 3 occasions
- Asked to come to the laboratories at IM Marsh campus (Mossley Hill, Liverpool) on 3 occasions to have some anthropometric measurements taken, have bloods taken and have their body composition measured via bioimpedance
- Asked to take a daily multivitamin and mineral supplement provided by us over the course of the whole intervention (very low-carb, high-fat group only)

***There are a number of exclusion criteria for the study, which are female, under the age of 19 or over the age of 64, non-White Caucasian, smoker, vegan/vegetarian, taking dietary supplements, having any known food allergies or intolerances, consuming alcohol above the weekly UK recommendations, suffering from an eating disorder, from current or previous renal impairment, having a history of cardiometabolic diseases, or taking lipid, blood pressure or blood glucose-lowering medication, BMI below 18.5 or over 30kg/m$^2$. Final eligibility will be based on obtaining a score between 4 and 11 in the following points system: fasting blood sugar concentration >5.5mmol/L = 3 points; waist >102cm or 40 inches = 2 points; waist >94 cm or 37 inches = 1 point; systolic blood pressure >130mmHg = 1 point; diastolic blood pressure >85mmHg = 1 point; HDL cholesterol concentration <1.0mmol/L = 2 points; serum triglyceride concentration >1.3mmol/L = 1 point. Dependent on the number of markers contributing to the potential participant's score this might mean that they are at increased risk of pre-metabolic or metabolic syndrome and potentially at increased risk of developing cardiovascular disease. Should the following thresholds found to have been exceeded during***

***practice). Although the finger prick method used is scientifically accurate, it might not be as precise as other methods used clinically and might differ from those obtained through a GP or a hospital setting. This means that our scoring system might deem the potential participant to be at increased risk of developing metabolic syndrome and cardiovascular disease when this is not the case. However, should they be concerned about any of the findings obtained during the screening process we would advise them to seek further advice from their GP.***

**4. Why do we need access to your facilities/staff/students?**

We are aiming to recruit 20 people (males) who are aged between 19 and 64 years and live in Liverpool. We think that your organisation could help us during the recruitment stage by forwarding the recruitment information to a number of people. We would greatly appreciate your support.

**5. If you are willing to assist in the study what happens next?**

We will arrange a time to visit your organisation that suits you best so that we can give you recruitment flyers should you wish to display them on your premises. In addition, we can send you a copy of the information via email.

**6. How we will use the Information?**

The information obtained from the study will be used to assess an individual's response to either a very low-carb, high-fat diet or a high-carb, moderate-fat (recommended in the UK dietary guidelines) diet and the impact on traditional and novel potential risk factors for cardiometabolic diseases such as heart disease and type 2 diabetes mellitus. This can inform future research and dietary recommendations in the general population and those at risk of cardiometabolic disease. In addition, each participant will receive a personalised dietary consultation at the end of the study based on their results (should they wish so).

**7. Will the name of my organisation taking part in the study be kept confidential?**

Completion of the study is voluntary; the information provided by you or anybody at your organisation will be unidentifiable in any publications. Any personal information collected during this study will be stored securely on password protected computers or in locked filing cabinets.

Should you have any comments or questions regarding this research, please do not hesitate to contact:

***Tanja Harrison, Faculty of Education, Health and Community***
***Contact details:*** *t.harrison@2015.ljmu.ac.uk*

***Dr Ian G. Davies, Faculty of Education, Health and Community***
***Contact details:*** *i.g.davies@ljmu.ac.uk*

**This study has received ethical approval from LJMU's Research Ethics Committee (16/ELS/029 approved 14 December 2016)**

***If you have any concerns regarding your involvement in this research, please discuss these with the researcher in the first instance. If you wish to make a complaint, please contact researchethics@ljmu.ac.uk and your communication will be re-directed to an independent person as appropriate.***

**Thank you for your time!**

## Gatekeeper Consent form

**CALIBER (Carbohydrates, lipids and biomarkers of traditional and emerging cardiometabolic risk factors) Phase 1: A pilot study in normal-weight and overweight adults**

Please tick to confirm your understanding of the study and that you are happy for your organisation to take part and your facilities to be used to host parts of the project.

*We would like to gain access to people who attend your organisation to invite them to take part in a research study is to investigate the effects of a very low-carb, high-fat or high-carb, moderate-fat (as recommended in the UK dietary guidelines) on cardiometabolic risk markers in normal-weight and overweight males and females aged 19-64 in a free-living situation.*

1. I confirm that I have read and understand the information provided for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that participation of our organisation and any of our employees/clients in the research is voluntary and that they are free to withdraw at any time, without giving a reason and that this will not affect legal rights. ☐
3. I understand that any personal information collected during the study will remain confidential. It will be anonymised for the purpose of publication. ☐
4. I agree for our organisation and any of our employees/clients to take part in the above study should they wish to do so. ☐
5. I understand that eligible individuals (as assessed via an online screening questionnaire and a finger prick to provide a small amount of blood) will be invited to complete an 8-week dietary intervention as outlined in the gatekeeper information sheet provided to me. ☐
6. I agree to conform to the data protection act ☐

Name of Gatekeeper: Date: Signature:

# Very low-carbohydrate, high-fat diet - Disclaimer

**CALIBER (Carbohydrates, lipids and biomarkers of traditional and emerging cardiometabolic risk factors) Phase 1: A pilot study in normal-weight and overweight adults**

I acknowledge that I have voluntarily chosen to participate in a dietary intervention consisting of two groups following either a very low-carb, high-fat diet or a high-carb, moderate-fat diet. The very low-carb, high-fat diet will contain very small amounts of available carbohydrates (minimum 30g per day and maximum 50g per day).

I certify that I have answered all questions on inclusion and exclusion criteria for the study truthfully.

Should I be allocated to the very low-carb, high-fat diet group I confirm that I understand that there are some adverse effects associated with very low-carb diets which are expected to last on average no longer than a week. These include

- Flu-like symptoms (headaches)
- Constipation through reduced fibre intake
- Bad breath
- Muscle cramps
- General weakness
- Impaired cognition or 'brain fog'
- Slight dizziness
- Mineral deficiencies
- Lack of vitamins

I understand that I will provided with information on how to best deal with these potential side effects and that I will be given detailed dietary guidance over the course of the intervention, including fibre-rich, magnesium and potassium-rich foods suitable for this diet. I will also be provided with a daily multivitamin and mineral

events sooner than this I can contact the researcher at any time under the contact details provided to me.

By signing this document I assume all responsibility for my health and well-being. I hold harmless of any responsibility the researcher, facility or any persons involved with this research study and testing procedures. I understand that I can ask questions at any time.

Name (participant)

Signature

Date

Name (witness)

Signature

Date

## Personal Details Form

**CALIBER (Carbohydrates, lipids and biomarkers of traditional and emerging cardiometabolic risk factors): A pilot study in normal-weight and overweight adult males**

If you have given your informed consent to participating in the study, we will need your contact details so we can keep in touch with you.

Your details will be kept in a secure, locked filing cabinet at Liverpool John Moores University. Only the researcher (Tanja Harrison) and the study's academic supervisor (Dr Ian Davies) will have access and your details will be kept separate from any data collected.

Your details will be kept for a maximum of 5 years after the study has concluded and will then be destroyed.

If you have any further questions regarding this, please do not hesitate to contact the researcher on the details outlined in the participant information sheet.

Please enter you contact details below: -

Name.....

Date of birth.....

Address.....

.....

.....

Postcode.....

Home telephone number.....

Mobile telephone number.....

# FOOD FREQUENCY QUESTIONNAIRE

This questionnaire asks for some background information about you, especially about what you eat.

Please answer every question. If you are uncertain about how to answer a question then do the best you can, but

1. **YOUR DIET LAST YEAR**

For each food there is an amount shown, either a "medium serving" or a common household unit such as a slice or teaspoon. Please put a tick (✓) in the box to indicate how often, **on average**, you have eaten the specified amount of each food **during the past year**.

**EXAMPLES:**

For white bread the amount is one slice, so if you ate 4 or 5 slices a day, you should put a tick in the column headed "4-5 per day".

| FOODS AND AMOUNTS | AVERAGE USE LAST YEAR | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **BREAD AND SAVOURY BISCUITS** (one slice or biscuit) | Never or less than once/month | 1-3 per month | Once a week | 2-4 per week | 5-6 per week | Once a day | 2-3 per day | 4-5 per day | 6+ per day |
| White bread and rolls | | | | | | | | ✓ | |

For chips, the amount is a "medium serving", so if you had a helping of chips twice a week you should put a tick in the column headed "2-4 per week".

| FOODS AND AMOUNTS | AVERAGE USE LAST YEAR | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **POTATOES, RICE AND PASTA** (medium serving) | Never or less than once/month | 1-3 per month | Once a week | 2-4 per week | 5-6 per week | Once a day | 2-3 per day | 4-5 per day | 6+ per day |
| Chips | | | | ✓ | | | | | |

For very seasonal fruits such as strawberries and raspberries you should estimate your average use when the fruits are in season, so if you ate strawberries or raspberries about once a week when they were in season you should put a tick in the column headed "once a week"

| FOODS AND AMOUNTS | AVERAGE USE LAST YEAR | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **FRUIT** (1 fruit or medium serving) | Never or less than once/month | 1-3 per month | Once a week | 2-4 per week | 5-6 per week | Once a day | 2-3 per day | 4-5 per day | 6+ per day |
| Strawberries, raspberries, kiwi fruit | | | ✓ | | | | | | |

**Please estimate your average food use as best you can, and please answer every question - do not leave ANY lines blank. PLEASE PUT A TICK (✓) ON EVERY LINE**

| FOODS AND AMOUNTS | AVERAGE USE LAST YEAR | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **MEAT AND FISH** (medium serving) | Never or less than once/month | 1-3 per month | Once a week | 2-4 per week | 5-6 per week | Once a day | 2-3 per day | 4-5 per day | 6+ per day |
| Beef: roast, steak, mince, stew or casserole | | | | | | | | | |
| Beefburgers | | | | | | | | | |
| Pork: roast, chops, stew or slices | | | | | | | | | |
| Lamb: roast, chops or stew | | | | | | | | | |
| Chicken or other poultry eg. turkey | | | | | | | | | |
| Bacon | | | | | | | | | |
| Ham | | | | | | | | | |
| Corned beef, Spam, luncheon meats | | | | | | | | | |
| Sausages | | | | | | | | | |
| Savoury pies, eg. meat pie, pork pie, pasties, steak & kidney pie, sausage rolls | | | | | | | | | |
| Liver, liver paté, liver sausage | | | | | | | | | |
| Fried fish in batter, as in fish and chips | | | | | | | | | |
| Fish fingers, fish cakes | | | | | | | | | |
| Other white fish, fresh or frozen, eg. cod, haddock, plaice, sole, halibut | | | | | | | | | |
| Oily fish, fresh or canned, eg. mackerel, kippers, tuna, salmon, sardines, herring | | | | | | | | | |
| Shellfish, eg. crab, prawns, mussels | | | | | | | | | |
| Fish roe, taramasalata | | | | | | | | | |
| | Never or less than once/month | 1-3 per month | Once a week | 2-4 per week | 5-6 per week | Once a day | 2-3 per day | 4-5 per day | 6+ per day |

**Please check that you have a tick (✓) on EVERY line**

PLEASE PUT A TICK (✓) ON EVERY LINE

| FOODS AND AMOUNTS | AVERAGE USE LAST YEAR | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **BREAD AND SAVOURY BISCUITS** (one slice or biscuit) | Never or less than once/month | 1-3 per month | Once a week | 2-4 per week | 5-6 per week | Once a day | 2-3 per day | 4-5 per day | 6+ per day |
| White bread and rolls | | | | | | | | | |
| Brown bread and rolls | | | | | | | | | |
| Wholemeal bread and rolls | | | | | | | | | |
| Cream crackers, cheese biscuits | | | | | | | | | |
| Crispbread, eg. Ryvita | | | | | | | | | |
| **CEREALS** (one bowl) | | | | | | | | | |
| Porridge, Readybrek | | | | | | | | | |
| Breakfast cereal such as cornflakes, muesli etc. | | | | | | | | | |
| **POTATOES, RICE AND PASTA** (medium serving) | | | | | | | | | |
| Boiled, mashed, instant or jacket potatoes | | | | | | | | | |
| Chips | | | | | | | | | |
| Roast potatoes | | | | | | | | | |
| Potato salad | | | | | | | | | |
| White rice | | | | | | | | | |
| Brown rice | | | | | | | | | |
| White or green pasta, eg. spaghetti, macaroni, noodles | | | | | | | | | |
| Wholemeal pasta | | | | | | | | | |
| Lasagne, moussaka | | | | | | | | | |
| Pizza | | | | | | | | | |
| | Never or less than once/month | 1-3 per month | Once a week | 2-4 per week | 5-6 per week | Once a day | 2-3 per day | 4-5 per day | 6+ per day |

**Please check that you have a tick (✓) on EVERY line**

PLEASE PUT A TICK (✓) ON EVERY LINE

| FOODS AND AMOUNTS | AVERAGE USE LAST YEAR | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **DAIRY PRODUCTS AND FATS** | Never or less than once/month | 1-3 per month | Once a week | 2-4 per week | 5-6 per week | Once a day | 2-3 per day | 4-5 per day | 6+ per day |
| Single or sour cream (tablespoon) | | | | | | | | | |
| Double or clotted cream (tablespoon) | | | | | | | | | |
| Low fat yogurt, fromage frais (125g carton) | | | | | | | | | |
| Full fat or Greek yogurt (125g carton) | | | | | | | | | |
| Dairy desserts (125g carton) | | | | | | | | | |
| Cheese, eg. Cheddar, Brie, Edam (medium serving) | | | | | | | | | |
| Cottage cheese, low fat soft cheese (medium serving) | | | | | | | | | |
| Eggs as boiled, fried, scrambled, etc. (one) | | | | | | | | | |
| Quiche (medium serving) | | | | | | | | | |
| Low calorie, low fat salad cream(tablespoon) | | | | | | | | | |
| Salad cream, mayonnaise (tablespoon) | | | | | | | | | |
| French dressing (tablespoon) | | | | | | | | | |
| Other salad dressing (tablespoon) | | | | | | | | | |
| **The following on bread or vegetables** | | | | | | | | | |
| Butter (teaspoon) | | | | | | | | | |
| Block margarine, eg. Stork, Krona (teaspoon) | | | | | | | | | |
| Polyunsaturated margarine (tub), eg. Flora, sunflower (teaspoon) | | | | | | | | | |
| Other soft margarine,dairy spreads (tub), eg. Blue Band, Clover (teaspoon) | | | | | | | | | |
| Low fat spread (tub), eg. Outline, Gold (teaspoon) | | | | | | | | | |
| Very low fat spread (tub) (teaspoon) | | | | | | | | | |
| | Never or less than once/month | 1-3 per month | Once a week | 2-4 per week | 5-6 per week | Once a day | 2-3 per day | 4-5 per day | 6+ per day |

**Please check that you have a tick (✓) on EVERY line**

PLEASE PUT A TICK (✓) ON EVERY LINE

| FOODS AND AMOUNTS | AVERAGE USE LAST YEAR | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **SWEETS AND SNACKS** (medium serving) | Never or less than once/month | 1-3 per month | Once a week | 2-4 per week | 5-6 per week | Once a day | 2-3 per day | 4-5 per day | 6+ per day |
| Sweet biscuits, chocolate , eg. digestive (one) | | | | | | | | | |
| Sweet biscuits, plain, eg. Nice, ginger (one) | | | | | | | | | |
| Cakes eg. fruit, sponge, home baked | | | | | | | | | |
| Cakes eg. fruit, sponge, ready made | | | | | | | | | |
| Buns, pastries eg. scones, flapjacks, home baked | | | | | | | | | |
| Buns, pastries eg. croissants, doughnuts, ready made | | | | | | | | | |
| Fruit pies, tarts, crumbles, home baked | | | | | | | | | |
| Fruit pies, tarts, crumbles, ready made | | | | | | | | | |
| Sponge puddings, home baked | | | | | | | | | |
| Sponge puddings, ready made | | | | | | | | | |
| Milk puddings, eg. rice, custard, trifle | | | | | | | | | |
| Ice cream, choc ices | | | | | | | | | |
| Chocolates, single or squares | | | | | | | | | |
| Chocolate snack bars eg. Mars, Crunchie | | | | | | | | | |
| Sweets, toffees, mints | | | | | | | | | |
| Sugar added to tea, coffee, cereal (teaspoon) | | | | | | | | | |
| Crisps or other packet snacks, eg. Wotsits | | | | | | | | | |
| Peanuts or other nuts | | | | | | | | | |
| **SOUPS, SAUCES, AND SPREADS** | | | | | | | | | |
| Vegetable soups (bowl) | | | | | | | | | |
| Meat soups (bowl) | | | | | | | | | |
| Sauces, eg. white sauce, cheese sauce, gravy (tablespoon) | | | | | | | | | |
| Tomato ketchup (tablespoon) | | | | | | | | | |
| Pickles, chutney (tablespoon) | | | | | | | | | |
| Marmite, Bovril (teaspoon) | | | | | | | | | |
| Jam, marmalade, honey (teaspoon) | | | | | | | | | |
| Peanut butter (teaspoon) | | | | | | | | | |
| | Never or less than once/month | 1-3 per month | Once a week | 2-4 per week | 5-6 per week | Once a day | 2-3 per day | 4-5 per day | 6+ per day |

**Please check that you have a tick (✓) on EVERY line**

PLEASE PUT A TICK (✓) ON EVERY LINE

| FOODS AND AMOUNTS | AVERAGE USE LAST YEAR | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **DRINKS** | Never or less than once/month | 1-3 per month | Once a week | 2-4 per week | 5-6 per week | Once a day | 2-3 per day | 4-5 per day | 6+ per day |
| Tea (cup) | | | | | | | | | |
| Coffee, instant or ground (cup) | | | | | | | | | |
| Coffee, decaffeinated (cup) | | | | | | | | | |
| Coffee whitener, eg. Coffee-mate (teaspoon) | | | | | | | | | |
| Cocoa, hot chocolate (cup) | | | | | | | | | |
| Horlicks, Ovaltine (cup) | | | | | | | | | |
| Wine (glass) | | | | | | | | | |
| Beer, lager or cider (half pint) | | | | | | | | | |
| Port, sherry, vermouth, liqueurs (glass) | | | | | | | | | |
| Spirits, eg. gin, brandy, whisky, vodka (single) | | | | | | | | | |
| Low calorie or diet fizzy soft drinks (glass) | | | | | | | | | |
| Fizzy soft drinks, eg. Coca cola, lemonade (glass) | | | | | | | | | |
| Pure fruit juice (100%) eg. orange, apple juice (glass) | | | | | | | | | |
| Fruit squash or cordial (glass) | | | | | | | | | |
| **FRUIT** **For seasonal fruits marked *, please estimate your average use when the fruit is in season** | | | | | | | | | |
| Apples (1 fruit) | | | | | | | | | |
| Pears (1 fruit) | | | | | | | | | |
| Oranges, satsumas, mandarins (1 fruit) | | | | | | | | | |
| Grapefruit (half) | | | | | | | | | |
| Bananas (1 fruit) | | | | | | | | | |
| Grapes (medium serving) | | | | | | | | | |
| Melon (1 slice) | | | | | | | | | |
| * Peaches, plums, apricots (1 fruit) | | | | | | | | | |
| * Strawberries, raspberries, kiwi fruit (medium serving) | | | | | | | | | |
| Tinned fruit (medium serving) | | | | | | | | | |
| Dried fruit, eg. raisins, prunes (medium serving) | | | | | | | | | |
| | Never or less than once/month | 1-3 per month | Once a week | 2-4 per week | 5-6 per week | Once a day | 2-3 per day | 4-5 per day | 6+ per day |

**Please check that you have a tick (✓) on EVERY line**

PLEASE PUT A TICK (✓) ON EVERY LINE

| FOODS AND AMOUNTS | AVERAGE USE LAST YEAR | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **VEGETABLES** Fresh, frozen or tinned (medium serving) | Never or less than once/month | 1-3 per month | Once a week | 2-4 per week | 5-6 per week | Once a day | 2-3 per day | 4-5 per day | 6+ per day |
| Carrots | | | | | | | | | |
| Spinach | | | | | | | | | |
| Broccoli, spring greens, kale | | | | | | | | | |
| Brussels sprouts | | | | | | | | | |
| Cabbage | | | | | | | | | |
| Peas | | | | | | | | | |
| Green beans, broad beans, runner beans | | | | | | | | | |
| Marrow, courgettes | | | | | | | | | |
| Cauliflower | | | | | | | | | |
| Parsnips, turnips, swedes | | | | | | | | | |
| Leeks | | | | | | | | | |
| Onions | | | | | | | | | |
| Garlic | | | | | | | | | |
| Mushrooms | | | | | | | | | |
| Sweet peppers | | | | | | | | | |
| Beansprouts | | | | | | | | | |
| Green salad, lettuce, cucumber, celery | | | | | | | | | |
| Watercress | | | | | | | | | |
| Tomatoes | | | | | | | | | |
| Sweetcorn | | | | | | | | | |
| Beetroot | | | | | | | | | |
| Coleslaw | | | | | | | | | |
| Avocado | | | | | | | | | |
| Baked beans | | | | | | | | | |
| Dried lentils, beans, peas | | | | | | | | | |
| Tofu , soya meat, TVP, Vegeburger | | | | | | | | | |
| | Never or less than once/month | 1-3 per month | Once a week | 2-4 per week | 5-6 per week | Once a day | 2-3 per day | 4-5 per day | 6+ per day |

**Please check that you have a tick (✓) on EVERY line**

**YOUR DIET LAST YEAR, continued**

2. Are there any **OTHER** foods which you ate more than once a week? Yes ☐ No ☐

**If yes,** please list below

| Food | Usual serving size | Number of times eaten each week |
|---|---|---|
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

3. What type of milk did you most often use?

**Select one only**

Full cream, silver ☐ Semi-skimmed, red/white ☐
Skimmed/blue ☐ Channel Islands, gold ☐
Dried milk ☐ Soya ☐
Other, *specify* ☐ None ☐

4. How much milk did you drink each day, including milk with tea, coffee, cereals etc?

None ☐ Three quarters of a pint ☐
Quarter of a pint ☐ One pint ☐
Half a pint ☐ More than one pint ☐

5. Did you usually eat breakfast cereal (excluding porridge and Ready Brek mentioned earlier)? Yes ☐ No ☐

**If yes,** which brand and type of breakfast cereal, including muesli, did you usually eat?

**List the one or two types most often used**

| Brand *e.g. Kellogg's* | Type *e.g. cornflakes* |
|---|---|
| | |
| | |

6. What kind of fat did you most often use for frying, roasting, grilling etc?

**Select one only**

Butter ☐ Solid vegetable fat ☐
Lard/dripping ☐ Margarine ☐
Vegetable oil ☐ None ☐

**If you used vegetable oil**, please give type eg. corn, sunflower ☐

7. What kind of fat did you most often use for baking cakes etc?

**Select one only**

Butter ☐ Solid vegetable fat ☐
Lard/dripping ☐ Margarine ☐
Vegetable oil ☐ None ☐

**If you used margarine**, please give name or type eg. Flora, Stork ☐

8. How often did you eat food that was fried at home?

Daily ☐ 1-3 times a week ☐ 4-6 times a week ☐
Less than once a week ☐ Never ☐

9. How often did you eat fried food away from home?

Daily ☐ 1-3 times a week ☐ 4-6 times a week ☐
Less than once a week ☐ Never ☐

10. What did you do with the visible fat on your meat?

Ate most of the fat ☐ Ate as little as possible ☐
Ate some of the fat ☐ Did not eat meat ☐

11. How often did you eat grilled or roast meat? ☐☐ times a week

12. How well cooked did you usually have grilled or roast meat?

Well done /dark brown ☐ Lightly cooked/rare ☐
Medium ☐ Did not eat meat ☐

13. How often did you add salt to food while cooking?

Always ☐ Rarely ☐
Usually ☐ Never ☐
Sometimes ☐

14. How often did you add salt to any food at the table?

Always ☐ Rarely ☐
Usually ☐ Never ☐
Sometimes ☐

15. Did you regularly use a salt substitute (eg LoSalt)? Yes ☐ No ☐

**If yes**, which brand? ☐

16. During the course of last year, on average, how many times a week did you eat the following foods?

| **Food type** | **Times/week** | **Portion size** |
|---|---|---|
| Vegetables (not including potatoes) | ☐☐ | medium serving |
| Salads | ☐☐ | medium serving |
| Fruit and fruit products (not including fruit juice) | ☐☐ | medium serving or 1 fruit |
| Fish and fish products | ☐☐ | medium serving |
| Meat, meat products and meat dishes (including bacon, ham and chicken) | ☐☐ | medium serving |

17. Have you taken any vitamins, minerals, fish oils, fibre or other food supplements during the past year? Yes ☐ No ☐ Don't know ☐

**If yes,** please complete the table below. If you have taken more than 5 types of supplement please put the most frequently consumed brands first.

| **Vitamin supplements** | **Average frequency** Tick **one** box per line to show how often on average you consumed supplements | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Name and brand** Please list full name, **brand** and strength | **Dose** Please state number of pills, capsules or teaspoons consumed | Never or less than once a month | 1-3 per month | Once a week | 2-4 per week | 5-6 per week | Once a day | 2-3 per day | 4-5 per day | 6+ per day |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |

*Thank you for your help*

MRC | Epidemiology Unit

Participant study No.

Available at http://www.mrc-epid.cam.ac.uk/Research/PA/Downloads.html

# RPAQ

Recent Physical Activity Questionnaire

This questionnaire is designed to find out about your physical activity in your everyday life in the last 4 weeks

**This questionnaire is divided into 3 sections**
Please try to answer every question.

- **Section A** asks about your physical activity patterns in and around the house.
- **Section B** is about travel to work and your activity at work.
- **Section C** asks about recreations that you may have engaged in during the last 4 weeks.

*Your answers will be treated as strictly confidential and will be used only for medical research*

## Section A Home Activities

**Getting about**

Which form of transport have you used **most often** in the last 4 weeks apart from your journey to and from work? (Please tick (✓) one box only)

| Usual mode of travel | | | |
|---|---|---|---|
| Car / motor vehicle | Walk | Public transport | Cycle |
| | | | |

**TV, DVD or Video Viewing** (Please put a tick (✓) on every line)

| Hours of TV, DVD or video watched per day | Average over the last 4 weeks | | | | | |
|---|---|---|---|---|---|---|
| | None | Less than 1 hour a day | 1 to 2 hours a day | 2 to 3 hours a day | 3 to 4 hours a day | More than 4 hours a day |
| On a weekday before 6 pm | | | | | | |
| On a weekday after 6 pm | | | | | | |
| On a weekend day before 6 pm | | | | | | |
| On a weekend day after 6 pm | | | | | | |

**Computer use at home *but not at work* (e.g. internet, email, Playstation, Xbox, Gameboy etc)** (Please put a tick (✓) on every line)

| Hours of home computer use per day | Average over the last 4 weeks | | | | | |
|---|---|---|---|---|---|---|
| | None | Less than 1 hour a day | 1 to 2 hours a day | 2 to 3 hours a day | 3 to 4 hours a day | More than 4 hours a day |
| On a weekday before 6 pm | | | | | | |
| On a weekday after 6 pm | | | | | | |
| On a weekend day before 6 pm | | | | | | |
| On a weekend day after 6 pm | | | | | | |

**Stair climbing at home** (please put a tick (✓) on every line)

| Number of times you climbed up a flight of stairs (approx 10 steps) each day at home | Average over the last 4 weeks | | | | | |
|---|---|---|---|---|---|---|
| | None | 1 to 5 times a day | 6 to 10 times a day | 11 to 15 times a day | 16 to 20 times a day | More than 20 times a day |
| On a weekday | | | | | | |
| On a weekend day | | | | | | |

## Section B Activity at work

Please answer this section to describe if you have been in paid employment at any time **during the last 4 weeks** or you have done regular, organised voluntary work.

Have you been in employment during the last 4 weeks? *Yes* ☐ *No* ☐

During the last 4 weeks how many hours work did you do per week?

| | 4 weeks ago | 3 weeks ago | 2 weeks ago | 1 week ago |
|---|---|---|---|---|
| Work hours (excluding travel) | | | | |

### Type of work

We would like to know the type and amount of physical activity involved in your work. **Please tick** (✓) the option that **best** corresponds with your occupation(s) in the last 4 weeks from the following four possibilities:

***Please tick only one of the following***

**1. Sedentary occupation**
You spend most of your time sitting (such as in an office) ☐

**2. Standing occupation**
You spend most of your time standing or walking. However, your work does not require intense physical effort (e.g. shop assistant, hairdresser, guard) ☐

**3. Manual work**
This involves some physical effort including handling of heavy objects and use of tools (e.g. plumber, electrician, carpenter) ☐

**4. Heavy manual work**
This implies very vigorous physical activity including handling of very heavy objects (e.g. dock worker, miner, bricklayer, construction worker) ☐

## Section B Activity at work

**Travel to and from work in the last 4 weeks**

What is the approximate distance from your home to your work?

☐☐☐ Miles *or* ☐☐☐ Kilometers

How many times a week did you travel from home to your main work? ☐☐
*Count outward journeys only*

Please tick (✓) one box **only** per line

| **How did you normally travel to work?** | Always | Usually | Occasionally | Never or rarely |
|---|---|---|---|---|
| By car/motor vehicle | | | | |
| By works or public transport | | | | |
| By bicycle | | | | |
| Walking | | | | |

What is the postcode for your main place of work during the last 4 weeks?

Postcode ☐☐☐☐☐☐☐

*If not known please give your work address*
Work address - _____
_____
_____

What is the postcode for your home address?

Postcode ☐☐☐☐☐☐☐

## Section C Recreation

The following questions ask about how you spent your leisure time.

Please indicate how often you did each activity on average over the last 4 weeks

Please indicate the average length of time that you spent doing the activity on each occasion.

**Example**

If you went walking for pleasure for 40 minutes once a week.
If you had done weeding or pruning every fortnight and took 1 hour and 10 minutes on each occasion.
You would complete the table below as follows:

**Please give an answer for the NUMBER OF TIMES you did the following activities in the past 4 weeks and the AVERAGE TIME you spent on each activity.**

**Please complete EACH line**

| | Number of times you did the activity in the last 4 weeks | | | | | | | Average time per episode | |
|---|---|---|---|---|---|---|---|---|---|
| | None | Once in the last 4 weeks | 2 to 3 times in the last 4 weeks | Once a week | 2 to 3 times a week | 4 to 5 times a week | Every day | Hours | Minutes |
| Weeding and pruning | | | ✓ | | | | | *1* | *10* |
| Walking for pleasure | | | | ✓ | | | | | *40* |



Now complete the table on pages 6 and 7

**Please give an answer for the average time you spent** on each activity and the number of times you did that activity in the past 4 weeks

**Please complete each line**

| | **Number of times you did the activity in the last 4 weeks** | | | | | | | Average time per episode | |
|---|---|---|---|---|---|---|---|---|---|
| | None | Once in the last 4 weeks | 2 to 3 times in the last 4 weeks | Once a week | 2 to 3 times a week | 4 to 5 times a week | Every day | Hours | Minutes |
| Swimming - competitive | | | | | | | | | |
| Swimming leisurely | | | | | | | | | |
| Backpacking or mountain climbing | | | | | | | | | |
| Walking for pleasure *(not as a means of transport)* | | | | | | | | | |
| Racing or rough terrain cycling | | | | | | | | | |
| Cycling for pleasure *(not as a means of transport)* | | | | | | | | | |
| Mowing the lawn | | | | | | | | | |
| Watering the lawn or garden | | | | | | | | | |
| Digging, shovelling or chopping wood | | | | | | | | | |
| Weeding or pruning | | | | | | | | | |
| DIY e.g. carpentry, home or car maintenance | | | | | | | | | |
| High impact aerobics or step aerobics | | | | | | | | | |
| Other types of aerobics | | | | | | | | | |
| Exercise with weights | | | | | | | | | |
| Conditioning exercises e.g. using a bike or rowing machine | | | | | | | | | |

**Please complete each line**

| | Number of times you did the activity in the last 4 weeks | | | | | | | Average time per episode | |
|---|---|---|---|---|---|---|---|---|---|
| | None | Once in the last 4 weeks | 2 to 3 times in the last 4 weeks | Once a week | 2 to 3 times a week | 4 to 5 times a week | Every day | Hours | Minutes |
| Floor exercises e.g. stretching, bending, keep fit or yoga | | | | | | | | | |
| Dancing e.g. ballroom or disco | | | | | | | | | |
| Competitive running | | | | | | | | | |
| Jogging | | | | | | | | | |
| Bowling- indoor, lawn or 10 pin | | | | | | | | | |
| Tennis or badminton | | | | | | | | | |
| Squash | | | | | | | | | |
| Table tennis | | | | | | | | | |
| Golf | | | | | | | | | |
| Football, rugby or hockey | | | | | | | | | |
| Cricket | | | | | | | | | |
| Rowing | | | | | | | | | |
| Netball, volleyball or basketball | | | | | | | | | |
| Fishing | | | | | | | | | |
| Horse-riding | | | | | | | | | |
| Snooker, billiards or darts | | | | | | | | | |
| Musical instrument playing or singing | | | | | | | | | |
| Ice skating | | | | | | | | | |
| Sailing, wind-surfing or boating | | | | | | | | | |
| Martial arts, boxing or wrestling | | | | | | | | | |

# CALIBER

Carbohydrates, lipids and biomarkers of traditional and emerging cardiometabolic risk factors

# 4-day FOOD AND DRINK DIARY

| For office use only | |
|---|---|
| Participant ID | Visit |
| | |

## Contents





Talmer: This diary follows the design used in the National Diet and Nutrition Survey (NDNS) Year 4 (2012)

## Instructions

**PLEASE READ THROUGH THESE PAGES BEFORE STARTING YOUR DIARY**

**We would like you to keep this diary of everything you eat and drink over 4 days. Please include all food consumed at home and outside the home e.g. work, university or restaurants.**

**When to fill in the diary**

**Please record your eating as you go, not from memory** at the end of the day. Use written notes on a pad if you forget to take your diary with you. Each diary day covers a 24hr period, so please include any food or drinks that you may have had during the night. Remember to include foods and drinks between meals (snacks) including water. This way it will only take a few minutes each time you complete parts of the diary.

**Day and Date**

Please write down the day and date at the top of the page each time you start a new day of recording. Please include one weekend day.

**Time Slots**

Please note the time of each eating occasion into the space provided. For easy use each day is divided into sections, from the first thing in the morning to late evening and through the night.

**Where and with whom?**

For each eating occasion, please tell us what **room or part of the house** you were in when you ate, e.g. kitchen, living room. If you ate at your work canteen, a restaurant, fast food chain or your car, write that location down. We would also like to know **who you share your meals with**, e.g. whether you ate alone or with others. If you ate with others please describe their relationship to you e.g. partner, children, colleagues, or friends. We would also like to know **when you ate at a table** and **when you were watching television whilst eating**. For those occasions where you were **not** at a table or watching TV please write 'Not at table' or 'No TV' rather than leaving it blank.

## What do you eat?

Please describe the food you eat in as much detail as possible. Be as specific as you can. Pages 3-10 will help with the sort of detail we need, like **cooking methods** (fried, grilled, baked etc) and any **additions** (fats, sugar/sweeteners, sauces, pepper etc).

- **Homemade dishes**

If you have eaten any **homemade dishes** e.g. chicken casserole, please record the name of the recipe, ingredients with amounts (including water or other fluids) for the whole recipe, the number of people the recipe serves, and the cooking method. Write this down in the recipe section at the end of the record day. Record how much of the whole recipe you have eaten in the portion size column (see examples in this booklet).
If you used a recipe provided in one of the CALIBER study menu plans, please just record [NAME OF RECIPE (CALIBER)], e.g. 'Chunky chilli con carne (CALIBER)'

- **Take-aways and eating out**

If you have eaten **take-aways** or **made up dishes not prepared at home** such as at a restaurant or a friend's house, please record as much detail about the ingredients as you can e.g. vegetable curry containing chickpeas, aubergine, onion and tomato.

- **Brand name**

Please note the **brand name** (if known). Most packed foods will list a brand name, e.g. Bird's eye, Hovis, or Supermarket own brands.

- ***Labels/Wrappers***

Labels are an important source of information for us. It helps us a great deal if you collect labels from all **ready meals,** and labels from **foods of lesser known brands** and bring them with you to your next lab appointment.
Or you could take a photo of the label and product name on your phone and email it to t.harrison@2015.llmu.ac.uk . Please state the date and eating occasion (breakfast, lunch, dinner, snack) when sending your photos. Thank you.

## Portion sizes

Examples for how to describe the **quantity** or **portion size** you had of a particular food or drink are shown further on in this booklet.
For foods, quantity can be described using:
- **household measures**, e.g. one teaspoon (tsp) of sugar, two thick slices of bread, 4 tablespoons (tbsp) of peas, ½ cup of gravy. Be careful when describing amounts in spoons that you are referring to the correct spoon size.
- **weights from labels**, e.g. 4oz steak, 420g tin of baked beans, 125g pot of yoghurt
- **number of items**, e.g. 4 fish fingers, 2 pieces of chicken nuggets, 1 regular size jam filled doughnut
- **picture examples** for specific foods can be found in this booklet.

For drinks, quantity can be described using:
- the **size of glass, cup etc** (e.g. large glass) or the **volume** (e.g. 300ml). Examples of typical drinks containers are on pages 15-16.
- **volumes from labels** (e.g. 330ml can of fizzy drink).

We would like to know the **amount that was actually eaten** which means taking **leftovers** into account. You can do this in two ways:
1. Record what was served and make notes of what was not eaten e.g. 3 tbsp of peas, only 2 tbsp eaten; 1 large sausage roll, ate only ½
2. Only record the amount actually eaten i.e. 2 tbsp of peas, ½ a large sausage roll

**Was it a typical day?**

After each day of recording you will be prompted to tell us whether this was a typical day or whether there were any reasons why you ate and drank more or less than usual.

***Please let us know if you would like an example of a completed food diary.***

## Examples and advice on food descriptions

| *Food Drink* | *Description & Preparation* | *Portion size or quantity* |
|---|---|---|
| Bacon | Back, middle, streaky; smoked or un-smoked; fat eaten; dry-fried or fried in oil/fat (type used) or grilled rashers | Number of rashers |
| Baked beans | Standard, reduced salt or reduced sugar | Spoons, weight of tin |
| Beefburger (hamburger) | Home-made (ingredients), from a packet or take-away; fried (type of oil/fat), microwaved or grilled; economy; with or without bread roll, with or without salad e.g. lettuce, tomato | Large or small, ounces or in grams if info on package |
| Beer | What sort e.g. stout, bitter, lager; draught, canned, bottled; % alcohol or low-alcohol or home-made | Number of pints or half pints, size of can or bottle |
| Biscuits | What sort e.g. cheese, wafer, crispbread, sweet, chocolate (fully or half coated), shortbread, home-made | Number, size (standard or mini variety) |
| Bread (see also sandwiches) | Wholemeal, granary, white or brown; currant, fruit, malt; large or small loaf; sliced or unsliced loaf | Number of slices; thick, medium or thin slices |
| Bread rolls | Wholemeal, white or brown; alone or with filling; crusty or soft | Size, number of rolls |
| Breakfast cereal (see also porridge) | What sort e.g. Kellogg's cornflakes; any added fruit and/or nuts; Muesli – with added fruit, no added sugar/salt variety | Spoons or picture 1 |
| Buns and pastries | What sort e.g. iced, currant or plain, jam, custard, fruit, cream; type of pastry; homemade or bought | Size, number |
| Butter, margarine & fat spreads | Give full product name | Thick/average/thin spread; spoons |
| Cake | What sort: fruit (rich), sponge, fresh cream, iced, chocolate coated; type of filling e.g. buttercream, jam | Individual or size of slice, packet weight, picture 10 |

| Food/Drink | Description & Preparation | Portion size or quantity |
|---|---|---|
| Cereal bars | What sort; with fruit/nuts, coated with chocolate/yoghurt; fortified with vitamins/minerals | Weight/size of bar; from multipack |
| Cheese | Type e.g. cheddar, cream, cottage, soft; low fat | Picture 9, or number of slices, number of spoons |
| Chips | Fresh, frozen, oven, microwave, take-away (where from); thick/straight/crinkle/fine cut; type of oil/fat used for cooking | Picture 4, as A, B, or C or 2 x B, etc |
| Chocolate(s) | What sort e.g. plain, milk, white, fancy, diabetic; type of filling; | Weight/size of bar |
| Coffee | With milk (see section on milk); half milk/half water; all milk; ground/filter, instant; decaffeinated. If café/takeaway, was it cappuccino, latte etc | Cups or mugs, size of takeaway e.g. small, medium |
| Cook-in sauces | What sort; pasta, Indian, Chinese, Mexican; tomato, white or cheese based; does meat or veg come in sauce; jar or can | Spoons, size of can or jar |
| Cream | Single, whipped, double or clotted; dairy or non-dairy; low-fat; fresh, UHT/Longlife; imitation cream e.g. Elmlea | Spoons |
| Crisps | What sort e.g. potato, corn, wheat, maize, vegetable etc; low-fat or low-salt; premium variety e.g. Kettle chips, Walker's Sensations | Packet weight, standard or from multipack |
| Custard | Pouring custard or egg custard; made with powder and milk/sugar, instant, ready to serve (tinned or carton); low fat, sugar free | Spoons |
| Egg | Boiled, poached, fried, scrambled, omelette (with or without filling); type of oil/fat, milk added | Number of eggs, large, medium or small |
| Fish (including canned) | What sort e.g. cod, tuna; fried (type of oil/fat), grilled, poached (water or milk) or steamed; with batter or breadcrumbs; canned in oil, brine or tomato sauce | Size of can or spoons (for canned fish) or picture 7 for battered fish |

| Food/Drink | Description & Preparation | Portion size or quantity |
|---|---|---|
| Fish cakes & fish fingers | Type of fish; plain or battered or in breadcrumbs; fried, grilled, baked or microwaved; economy | Size, number, packet weight |
| Fruit - fresh | What sort; eaten with or without skin | Small, medium or large |
| Fruit - stewed/canned | What sort; sweetened or unsweetened; in fruit juice or syrup; juice or syrup eaten | Spoons, weight of can |
| Fruit – juice (pure) | What sort e.g. apple, orange; sweetened or unsweetened; pasteurised or UHT/Longlife; freshly squeezed; added vitamins/minerals, omega 3 | Glass (size or volume) or carton size |
| Ice cream | Flavour; dairy or non-dairy alternatives e.g. soya; luxury/premium | Spoons/ scoops |
| Jam, honey | What sort; low-sugar/diabetic; shop bought/brand or homemade | Spoons, heaped or level, or thin or thick spread |
| Marmalade | Type; low-sugar; thick cut; shop bought/brand or homemade | Spoons, heaped or level, or thin or thick spread |
| Meat (see also bacon, burgers & sausages) | What sort; cut of meat e.g. chop, breast, minced; lean or fatty; fat removed or eaten; skin removed or eaten; how cooked; with or without gravy | Large/small/medium, spoons, or picture 6 for stew portion |
| Milk | What sort; whole, semi-skimmed, skimmed or 1% fat; fresh, sterilized, UHT, dried; soya milk (sweetened/unsweetened), goats' milk, rice milk, oat milk; flavoured; fortified with added vitamins and/or minerals | Pints, glass (size or volume) or cup. On cereal: *damp/normal/ drowned*. In tea/coffee: *a little/some/a lot* |

| Food/Drink | Description & Preparation | Portion size or quantity |
|---|---|---|
| Nuts | What sort; dry roasted, ordinary salted, honey roasted; unsalted | Packet weight, handful |
| Pie (sweet or savoury) | What sort/filling; one pastry crust or two; type of pastry | Individual or slice, or picture 8 |
| Pizza | Thin base/deep pan or French bread; topping e.g. meat, fish, veg; stuffed crust | Individual, slice, fraction of large pizza e.g. ¼ |
| Porridge | Made with oats or cornmeal or instant oat cereal; made with milk and/or water; added sugar, honey, syrup or salt; with milk or cream | Bowls, spoons |
| Potatoes (see also chips) | Old or new; baked, boiled, roast (type of oil/fat); skin eaten; mashed (with butter/spread and with or without milk); fried/chips (type of oil/fat); instant; any additions e.g. butter | Mash – spoons, number of half or whole potatoes, small or large potatoes |
| Pudding | What sort; e.g. steamed sponge; with fruit; mousse; instant desserts; milk puddings | Spoons, picture 10 for slice of sponge |
| Rice | What sort; e.g. basmati, easy cook, long or short grain; white or brown; boiled or fried (type of oil/fat) | Spoons or picture 2 |
| Salad | Ingredients; if with dressing what sort (oil and vinegar, mayonnaise) | Amount of each component |
| Sandwiches and rolls | Type of bread/roll (see Bread & Rolls); butter or margarine; type of filling; including salad, mayonnaise, pickle etc. If shop-bought, where from? | Number of rolls or slices of bread; amount of butter/margarine (on both slices?); amount of filling |
| Sauce – cold (including mayonnaise) | Tomato ketchup, brown sauce, soy sauce, salad cream, mayonnaise; low fat; | Spoons |

| *Food/Drink* | *Description & Preparation* | *Portion size or quantity* |
|---|---|---|
| Sauce – hot (see also cook-in sauces) | What sort; savoury or sweet; thick or thin; for gravy - made with granules, stock cube, dripping or meat juices | Spoons |
| Sausages | What sort; e.g. beef, pork; fried (type of oil/fat) or grilled; low fat | Large or small, number |
| Sausage rolls | Type of pastry | Size - jumbo, standard, mini |
| Scone | Fruit, sweet, plain, cheese; type of flour; homemade | Small, medium or large |
| Savoury snacks - in packet | What sort: e.g. Cheddars, cheese straws, Twiglets, Pretzels | Size (standard or mini variety), packet weight |
| Smoothies | If homemade give recipe. If shop-bought, what does it contain e.g. fruit, milk/yoghurt, fruit juice | Glass or bottle (size or volume) |
| Soft drinks – squash/ concentrate/cordial | Flavour; no added sugar/low calorie/sugar free; "high" juice; fortified with added vitamins and/or minerals | Glass (size or volume) |
| Soft drinks – carbonated/fizzy | Flavour; diet/low-calorie; canned or bottled; cola – caffeine free | Glass, can or bottle (size or volume) |
| Soft drinks – ready to drink | Flavour; no added sugar/low calorie/sugar free; real fruit juice? If so, how much?; fortified with added vitamins and/or minerals | Glass, carton or bottle (size or volume) |
| Soup | What sort; cream or clear; fresh/chilled, canned, instant or vending machine. If home-made, give recipe | Spoons, bowl or mug |
| Spaghetti, other pasta | What sort; fresh/chilled or dried; white, wholemeal; canned in sauce; type of filling if ravioli, cannelloni etc | Spoons (or how much dry pasta) or picture 3 |

| *Food/Drink* | *Description & Preparation* | *Portion size or quantity* |
|---|---|---|
| Spirits | What sort: e.g. whisky, gin, vodka, rum | Measures as in pub |
| Sugar | Added to cereals, tea, coffee, fruit, etc; what sort; e.g. white, brown, demerara | Heaped or level teaspoons |
| Sweets | What sort: e.g. toffees, boiled sweets, diabetic, sugar-free | Number, packet weight |
| Tea | With/without milk (see section on milk); decaffeinated, herb | Mugs or cups |
| Vegetables (not including potatoes) | What sort; how cooked/raw; additions e.g. butter, other fat or sauce | Spoons, number of florets or sprouts, weight from tins or packet |
| Wine, sherry, port | White, red; sweet, dry; % alcohol or low-alcohol | Glass (size or volume) |
| Yoghurt (inc drinking yoghurt), fromage frais | What sort: e.g. natural/plain or flavoured; creamy, Greek, low-fat, very low fat/diet, soya; with fruit pieces or fruit flavoured; twinpot; fortified with added vitamins and/or minerals; longlife/UHT; probiotic | Pot size or spoons |
| Home-made dishes | Please say what the dish is called (record recipe or details of dish if you can in the section provided) and how many persons it serves | Spoons – heaped or level, number, size |
| Ready-made meals | Full description of product; does it contain any accompaniments e.g. rice, vegetables, sauces; chilled or frozen; microwaved, oven cooked, boil-in-the-bag; low fat, healthy eating range. Enclose label and ingredients list if possible in your plastic bag | Packet weight (if didn't eat whole packet describe portion consumed) |
| Take-away food or food eaten out | Please say what the dish is called and give main ingredients if you can. Give name of a chain restaurant e.g. McDonalds | Spoons, portion size e.g. small/medium/large |

## Pictures for food portion size guidance

**Use the pictures to help you indicate the size of the portion you have eaten.**
**Write on the food record the picture number and size A, B or C nearest to your own helping.**

**Remember that the pictures are much smaller than life size.**
**The actual size of the dinner plate is 10 inches (25cm), the side plate, 7 inches (18cm), and the bowl, 6.3 inches (16cm).**

1. Breakfast cereals



2. Rice


3. Spaghetti


4. Chips

5. Broccoli/ cauliflower


6. Stew /curry


7. Battered fish

8. Quiche / Pie


9. Cheese


10. Sponge cake

## Drink volume guidance - Typical quantities of drinks in various containers measured in millimetres (ml)

| | Small glass | Average (medium) glass | Large glass | Vending cup | Cup | Mug |
|---|---|---|---|---|---|---|
| Soft drinks | 150 | 200 | 300 | | | |
| Wine | 125 | 175 | 250 | | | |
| Hot drinks | | | | 170 | 190 | 260 |

***Glasses come in different shapes and sizes. On the next page is a life size glass showing approximate volumes. You can use this to estimate how much you have consumed.***

**Life Size Glass**


27

# The 4-day diary

| Day 1: | | Date: | | |
|---|---|---|---|---|
| Time | Where? With whom? TV on? At table? | Food/Drink description & preparation | Brand name | Portion size and quantity eaten (e.g. half of it/all of it etc.) |
| 6am to 9am | | | | |
| | | | | |
| 9am to 12 noon | | | | |
| | | | | |

| Time | Where? With whom? TV on? At table? | Food/Drink description & preparation | Brand name | Portion size and quantity eaten (e.g. half of it/all of it etc.) |
|---|---|---|---|---|
| 12 noon to 2pm | | | | |
| | | | | |
| 2pm to 5pm | | | | |
| | | | | |

| Time | Where? With whom? TV on? At table? | Food/Drink description & preparation | Brand name | Portion size and quantity eaten (e.g. half of it/all of it etc.) |
|---|---|---|---|---|
| 5pm to 8pm | | | | |
| | | | | |
| 8pm to 10pm | | | | |
| | | | | |
| 10pm to 6am | | | | |
| | | | | |

Was the amount of **food** that you had today about what you usually have, less than usual, or more than usual?

Yes, usual ☐ No, **less** than usual ☐ No, **more** than usual ☐

*Please tell us why you had less than usual*

*Please tell us why you had more than usual*

Was the amount you had to **drink** today, including water, tea, coffee and soft drinks [and alcohol], about what you usually have, less than usual, or more than usual?

Yes, usual ☐ No, **less** than usual ☐ No, **more** than usual ☐

*Please tell us why you had less than usual*

*Please tell us why you had more than usual*

Did you **finish all the food and drink** that you recorded in the diary today?

Yes ☐ No ☐

If no, please **go back to the diary and make a note of any leftovers**

**Please record on the next pages any recipes or (if not already described) ingredients of made up dishes or take-away dishes.**

| Write in recipes or ingredients of made up dishes or take-away dishes | | | |
|---|---|---|---|
| *NAME OF DISH:* | | *Serves:* | |
| *Ingredients* | *Amount* | *Ingredients* | *Amount* |
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| | | | |
| *Brief description of cooking method* | | | |

| Write in recipes or ingredients of made up dishes or take-away dishes | | | |
|---|---|---|---|
| *NAME OF DISH:* | | *Serves:* | |
| *Ingredients* | *Amount* | *Ingredients* | *Amount* |
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| *Brief description of cooking method* | | | |

| Day 2: | | Date: | | |
|---|---|---|---|---|
| Time | Where? With whom? TV on? At table? | Food/Drink description & preparation | Brand name | Portion size and quantity eaten (e.g. half of it/all of it etc.) |
| 6am to 9am | | | | |
| | | | | |
| 9am to 12 noon | | | | |
| | | | | |

| Time | Where? With whom? TV on? At table? | Food/Drink description & preparation | Brand name | Portion size and quantity eaten (e.g. half of it/all of it etc.) |
|---|---|---|---|---|
| 12 noon to 2pm | | | | |
| | | | | |
| 2pm to 5pm | | | | |
| | | | | |

| Time | Where? With whom? TV on? At table? | Food/Drink description & preparation | Brand name | Portion size and quantity eaten (e.g. half of it/all of it etc.) |
|---|---|---|---|---|
| 5pm to 8pm | | | | |
| | | | | |
| 8pm to 10pm | | | | |
| | | | | |
| 10pm to 6am | | | | |
| | | | | |

Was the amount of **food** that you had today about what you usually have, less than usual, or more than usual?

Yes, usual ☐ No, **less** than usual ☐ No, **more** than usual ☐

*Please tell us why you had less than usual*

*Please tell us why you had more than usual*

Was the amount you had to **drink** today, including water, tea, coffee and soft drinks [and alcohol], about what you usually have, less than usual, or more than usual?

Yes, usual ☐ No, **less** than usual ☐ No, **more** than usual ☐

*Please tell us why you had less than usual*

*Please tell us why you had more than usual*

Did you **finish all the food and drink** that you recorded in the diary today?

Yes ☐ No ☐

If no, please **go back to the diary and make a note of any leftovers**

**Please record on the next pages any recipes or (if not already described) ingredients of made up dishes or take-away dishes.**

| Write in recipes or ingredients of made up dishes or take-away dishes | | | |
|---|---|---|---|
| *NAME OF DISH:* | | *Serves:* | |
| *Ingredients* | *Amount* | *Ingredients* | *Amount* |
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| | | | |
| *Brief description of cooking method* | | | |

| Write in recipes or ingredients of made up dishes or take-away dishes | | | |
|---|---|---|---|
| *NAME OF DISH:* | | *Serves:* | |
| *Ingredients* | *Amount* | *Ingredients* | *Amount* |
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| *Brief description of cooking method* | | | |

| Day 3: | | Date: | | |
|---|---|---|---|---|
| Time | Where? With whom? TV on? At table? | Food/Drink description & preparation | Brand name | Portion size and quantity eaten (e.g. half of it/all of it etc.) |
| 6am to 9am | | | | |
| | | | | |
| 9am to 12 noon | | | | |
| | | | | |

| Time | Where? With whom? TV on? At table? | Food/Drink description & preparation | Brand name | Portion size and quantity eaten (e.g. half of it/all of it etc.) |
|---|---|---|---|---|
| 12 noon to 2pm | | | | |
| | | | | |
| 2pm to 5pm | | | | |
| | | | | |

| Time | Where? With whom? TV on? At table? | Food/Drink description & preparation | Brand name | Portion size and quantity eaten (e.g. half of it/all of it etc.) |
|---|---|---|---|---|
| 5pm to 8pm | | | | |
| | | | | |
| 8pm to 10pm | | | | |
| | | | | |
| 10pm to 6am | | | | |
| | | | | |

Was the amount of **food** that you had today about what you usually have, less than usual, or more than usual?

Yes, usual ☐ No, **less** than usual ☐ No, **more** than usual ☐

*Please tell us why you had less than usual*

*Please tell us why you had more than usual*

Was the amount you had to **drink** today, including water, tea, coffee and soft drinks [and alcohol], about what you usually have, less than usual, or more than usual?

Yes, usual ☐ No, **less** than usual ☐ No, **more** than usual ☐

*Please tell us why you had less than usual*

*Please tell us why you had more than usual*

Did you **finish all the food and drink** that you recorded in the diary today?

Yes ☐ No ☐

If no, please **go back to the diary and make a note of any leftovers**

**Please record on the next pages any recipes or (if not already described) ingredients of made up dishes or take-away dishes.**

| Write in recipes or ingredients of made up dishes or take-away dishes | | | |
|---|---|---|---|
| *NAME OF DISH:* | | *Serves:* | |
| *Ingredients* | *Amount* | *Ingredients* | *Amount* |
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| | | | |
| *Brief description of cooking method* | | | |

| Write in recipes or ingredients of made up dishes or take-away dishes | | | |
|---|---|---|---|
| *NAME OF DISH:* | | *Serves:* | |
| *Ingredients* | *Amount* | *Ingredients* | *Amount* |
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| | | | |
| *Brief description of cooking method* | | | |

| Day 4: | | Date: | | |
|---|---|---|---|---|
| Time | Where? With whom? TV on? At table? | Food/Drink description & preparation | Brand name | Portion size and quantity eaten (e.g. half of it/all of it etc.) |
| 6am to 9am | | | | |
| | | | | |
| 9am to 12 noon | | | | |
| | | | | |

| Time | Where? With whom? TV on? At table? | Food/Drink description & preparation | Brand name | Portion size and quantity eaten (e.g. half of it/all of it etc.) |
|---|---|---|---|---|
| 12 noon to 2pm | | | | |
| | | | | |
| 2pm to 5pm | | | | |
| | | | | |

| Time | Where? With whom? TV on? At table? | Food/Drink description & preparation | Brand name | Portion size and quantity eaten (e.g. half of it/all of it etc.) |
|---|---|---|---|---|
| 5pm to 8pm | | | | |
| | | | | |
| 8pm to 10pm | | | | |
| | | | | |
| 10pm to 6am | | | | |
| | | | | |

Was the amount of **food** that you had today about what you usually have, less than usual, or more than usual?

Yes, usual ☐ No, **less** than usual ☐ No, **more** than usual ☐

*Please tell us why you had less than usual*

*Please tell us why you had more than usual*

Was the amount you had to **drink** today, including water, tea, coffee and soft drinks [and alcohol], about what you usually have, less than usual, or more than usual?

Yes, usual ☐ No, **less** than usual ☐ No, **more** than usual ☐

*Please tell us why you had less than usual*

*Please tell us why you had more than usual*

Did you **finish all the food and drink** that you recorded in the diary today?

Yes ☐ No ☐

If no, please **go back to the diary and make a note of any leftovers**

**Please record on the next pages any recipes or (if not already described) ingredients of made up dishes or take-away dishes.**

| Write in recipes or ingredients of made up dishes or take-away dishes | | | |
|---|---|---|---|
| *NAME OF DISH:* | | *Serves:* | |
| *Ingredients* | *Amount* | *Ingredients* | *Amount* |
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| *Brief description of cooking method* | | | |

| Write in recipes or ingredients of made up dishes or take-away dishes | | | |
|---|---|---|---|
| *NAME OF DISH:* | | *Serves:* | |
| *Ingredients* | *Amount* | *Ingredients* | *Amount* |
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| *Brief description of cooking method* | | | |

Check list of fibre-rich foods consumed during intervention

**CALIBER (Carbohydrates, lipids and biomarkers of traditional and emerging cardiometabolic risk factors): A pilot study in normal-weight and overweight adult males**

**Participant ID** **Date**

Over the past 4 weeks, did you consume any of the following foods?

*Tick all that apply*

| Food name | Consumed |
|---|---|
| Almonds | |
| Apples | |
| Asparagus | |
| Aubergines | |
| Avocado | |
| Bananas | |
| Beans, baked in tomato sauce (tinned) | |
| Beans, broad | |
| Beans, butter | |
| Beans, green | |
| Beans, red kidney | |
| Beans, soy/edamame | |
| Beetroot | |
| Blackberries | |
| Brazil nuts | |
| Broccoli | |
| Broccoli, Purple sprouting | |
| Brussel sprouts | |
| Butternut squash | |
| Cabbage, green | |
| Cabbage, red | |

| | |
|---|---|
| Chickpeas, canned, re-heated | |
| Coconut, desiccated | |
| Coconut, fresh | |
| Courgette | |
| Cucumber | |
| Fennel, Florence | |
| Flaxseeds (Linseeds) | |
| Hazelnuts | |
| High fibre breakfast cereal | |
| Kale, curly | |
| Kohlrabi | |
| Leeks | |
| Lentils, green or brown | |
| Lentils, red | |
| Lettuce, cos | |
| Lettuce, iceberg | |
| Lettuce, romaine | |
| Mushrooms, oyster | |
| Mushrooms, Portobello | |
| Mushrooms, shitake | |
| Mushrooms, white | |
| Oat bran | |
| Olives, green in brine | |
| Onions | |
| Oranges | |
| Parsley | |
| Parsnip | |
| Peanuts | |
| Peanut butter, smooth | |
| Pears | |
| Peas, green | |
| Pecan nuts | |
| Pepper, green | |

| | |
|---|---|
| Pumpkin | |
| Pumpkin seeds | |
| Radishes | |
| Raspberries, red | |
| Rocket | |
| Spinach, baby | |
| Spinach, frozen | |
| Spring onion | |
| Strawberries | |
| Sunflower seeds | |
| Sweetcorn | |
| Sweet potato | |
| Tomatoes | |
| Turnips | |
| Walnuts | |
| Watercress | |
| Wholegrain bread | |
| Wholegrain pasta | |

# UK Food Cravings Inventory Questionnaire

## Baseline

Date:

Food craving is defined as an intense desire to consume a particular food (or food type) that is difficult to resist.

*Directions:* For each of the foods listed below (Items 1 – 24), please circle the appropriate letter using the following scale.

Over the past month how often have you experienced a craving for the food?

| | | Always /Almost every day | Often | Sometimes | Rarely | Never |
|---|---|---|---|---|---|---|
| 1. | **Fast food** | 4 | 3 | 2 | 1 | 0 |
| 2. | **French fries** | 4 | 3 | 2 | 1 | 0 |
| 3. | **Burger** | 4 | 3 | 2 | 1 | 0 |
| 4. | **Pizza** | 4 | 3 | 2 | 1 | 0 |
| 5. | **Fried chicken** | 4 | 3 | 2 | 1 | 0 |
| 6. | **Cake** | 4 | 3 | 2 | 1 | 0 |
| 7. | **Chocolate** | 4 | 3 | 2 | 1 | 0 |
| 8. | **Biscuits** | 4 | 3 | 2 | 1 | 0 |
| 9. | **Ice cream** | 4 | 3 | 2 | 1 | 0 |
| 10. | **Popcorn** | 4 | 3 | 2 | 1 | 0 |
| 11. | **Sweets** | 4 | 3 | 2 | 1 | 0 |
| 12. | **Ice lolly** | 4 | 3 | 2 | 1 | 0 |
| 13. | **Sausage** | 4 | 3 | 2 | 1 | 0 |
| 14. | **Hotdog** | 4 | 3 | 2 | 1 | 0 |

| | | Always /Almost every day | Often | Sometimes | Rarely | Never |
|---|---|---|---|---|---|---|
| 15. | Bacon | 4 | 3 | 2 | 1 | 0 |
| 16. | Steak | 4 | 3 | 2 | 1 | 0 |
| 17. | Gravy | 4 | 3 | 2 | 1 | 0 |
| 18. | Pasta | 4 | 3 | 2 | 1 | 0 |
| 19. | Baked potato | 4 | 3 | 2 | 1 | 0 |
| 20. | Curry | 4 | 3 | 2 | 1 | 0 |
| 21. | Rice | 4 | 3 | 2 | 1 | 0 |
| 22. | Mashed Potato | 4 | 3 | 2 | 1 | 0 |
| 23. | Pasty | 4 | 3 | 2 | 1 | 0 |
| 24. | Bread | 4 | 3 | 2 | 1 | 0 |

Of these times in the past month during which you craved a particular food, how often did you "give in" to the craving and eat the food?

| | | Always /Almost every time | Often | Sometimes | Rarely (Once or twice) | Never |
|---|---|---|---|---|---|---|
| 1. | Fast food | 4 | 3 | 2 | 1 | 0 |
| 2. | French fries | 4 | 3 | 2 | 1 | 0 |
| 3. | Burger | 4 | 3 | 2 | 1 | 0 |
| 4. | Pizza | 4 | 3 | 2 | 1 | 0 |
| 5. | Fried chicken | 4 | 3 | 2 | 1 | 0 |
| 6. | Cake | 4 | 3 | 2 | 1 | 0 |
| 7. | Chocolate | 4 | 3 | 2 | 1 | 0 |
| 8. | Biscuits | 4 | 3 | 2 | 1 | 0 |
| 9. | Ice cream | 4 | 3 | 2 | 1 | 0 |
| 10. | Popcorn | 4 | 3 | 2 | 1 | 0 |
| 11. | Sweets | 4 | 3 | 2 | 1 | 0 |
| 12. | Ice lolly | 4 | 3 | 2 | 1 | 0 |

| | | Always /Almost every time | Often | Sometimes | Rarely (Once or twice) | Never |
|---|---|---|---|---|---|---|
| 13. | Sausage | 4 | 3 | 2 | 1 | 0 |
| 14. | Hotdog | 4 | 3 | 2 | 1 | 0 |
| 15. | Bacon | 4 | 3 | 2 | 1 | 0 |
| 16. | Steak | 4 | 3 | 2 | 1 | 0 |
| 17. | Gravy | 4 | 3 | 2 | 1 | 0 |
| 18. | Pasta | 4 | 3 | 2 | 1 | 0 |
| 19. | Baked potato | 4 | 3 | 2 | 1 | 0 |
| 20. | Curry | 4 | 3 | 2 | 1 | 0 |
| 21. | Rice | 4 | 3 | 2 | 1 | 0 |
| 22. | Mashed Potato | 4 | 3 | 2 | 1 | 0 |
| 23. | Pasty | 4 | 3 | 2 | 1 | 0 |
| 24. | Bread | 4 | 3 | 2 | 1 | 0 |

How difficult was it to resist temptation?

| | | Very difficult | Difficult | Neutral | Easy | Very easy |
|---|---|---|---|---|---|---|
| 1. | Fast food | 4 | 3 | 2 | 1 | 0 |
| 2. | French fries | 4 | 3 | 2 | 1 | 0 |
| 3. | Burger | 4 | 3 | 2 | 1 | 0 |
| 4. | Pizza | 4 | 3 | 2 | 1 | 0 |
| 5. | Fried chicken | 4 | 3 | 2 | 1 | 0 |
| 6. | Cake | 4 | 3 | 2 | 1 | 0 |
| 7. | Chocolate | 4 | 3 | 2 | 1 | 0 |
| 8. | Biscuits | 4 | 3 | 2 | 1 | 0 |
| 9. | Ice cream | 4 | 3 | 2 | 1 | 0 |
| 10. | Popcorn | 4 | 3 | 2 | 1 | 0 |
| 11. | Sweets | 4 | 3 | 2 | 1 | 0 |

| | | Very difficult | Difficult | Neutral | Easy | Very easy |
|---|---|---|---|---|---|---|
| 12. | Ice lolly | 4 | 3 | 2 | 1 | 0 |
| 13. | Sausage | 4 | 3 | 2 | 1 | 0 |
| 14. | Hotdog | 4 | 3 | 2 | 1 | 0 |
| 15. | Bacon | 4 | 3 | 2 | 1 | 0 |
| 16. | Steak | 4 | 3 | 2 | 1 | 0 |
| 17. | Gravy | 4 | 3 | 2 | 1 | 0 |
| 18. | Pasta | 4 | 3 | 2 | 1 | 0 |
| 19. | Baked potato | 4 | 3 | 2 | 1 | 0 |
| 20. | Curry | 4 | 3 | 2 | 1 | 0 |
| 21. | Rice | 4 | 3 | 2 | 1 | 0 |
| 22. | Mashed Potato | 4 | 3 | 2 | 1 | 0 |
| 23. | Pasty | 4 | 3 | 2 | 1 | 0 |
| 24. | Bread | 4 | 3 | 2 | 1 | 0 |

## WEAR TIME DIARY

Please use the table below to record A) The time you put the monitor on and take it off each day; B) Reasons why you took the monitor off during the day

| Date | Day | Time you put the monitor on (HH:MM) | Time you took the monitor off (HH:MM) | Reason for taking monitor off |
|---|---|---|---|---|
| 4th May | Monday | 06:15 | 22:30 | went to bed |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

**If you have any questions please contact:** Tanja Harrison: t.harrison@2015.ljmu.ac.uk 07970 858 594

*Pre-study*

*Office use only: Participant ID:*

LIVERPOOL JOHN MOORES UNIVERSITY

# ACTIVITY MONITOR GUIDE

## Where do I wear the monitor?

- Wear the monitor strapped securely to your non-dominant wrist with the logo facing up
- You can use the monitor to tell the time, just like a wrist-watch
- Tighten the strap enough so the monitor does not move when you are moving

## When do I wear it?

- **All the time you are awake for the next 7 days**
- Put it on first thing in the morning and take it off just before you go to bed
- You should be wearing the monitor for *at least* 10 hours each day
- Please do not wear the monitor during water-based activities, such as swimming, showering or bathing, or when playing sports/engaging in activities where watches are prohibited

## When and how do I give the monitor back?

A member of the research team will collect the monitor from you at

## Adverse events (AE) structured 4-weekly interview

**CALIBER (Carbohydrates, lipids and biomarkers of traditional and emerging cardiometabolic risk factors): A pilot study in normal-weight and overweight adult males**

Participant ID

Date

Carbohydrate phase over previous 4 week period (please tick as appropriate):

Very low ☐ Low ☐

Moderate ☐ High ☐

Over the past 4 weeks have you experienced any of the following side effects that you would say were a direct result of your participation in this study, such as

| **Impaired cognition/'brain fog'** | Yes ☐ | No ☐ |
|---|---|---|
| Do you remember on which date (or thereabout) this occurred? | | |
| Can you give further details of what happened? | | |
| Can you remember how long this lasted? | | |
| How severe would you say these effects were? | | |
| What did you do to ameliorate these effects? | | |

| **Dizziness** | Yes ☐ | No ☐ |
|---|---|---|
| Do you remember on which date (or thereabout) this occurred? | | |
| Can you give further details of what happened? | | |
| Can you remember how long this lasted? | | |
| How severe would you say these effects were? | | |
| What did you do to ameliorate these effects? | | |

| **Constipation** | Yes ☐ | No ☐ |
|---|---|---|
| Do you remember on which date (or thereabout) this occurred? | | |
| Can you give further details of what happened? | | |
| Can you remember how long this lasted? | | |
| How severe would you say these effects were? | | |
| What did you do to ameliorate these effects? | | |

| **Headaches or other flu-like symptoms** | Yes ☐ | No ☐ |
|---|---|---|
| Do you remember on which date (or thereabout) this occurred? | | |
| Can you give further details of what happened? | | |
| Can you remember how long this lasted? | | |
| How severe would you say | | |

| **Muscle cramps** | Yes ☐ | No ☐ |
|---|---|---|
| Do you remember on which date (or thereabout) this occurred? | | |
| Can you give further details of what happened? | | |
| Can you remember how long this lasted? | | |
| How severe would you say these effects were? | | |
| What did you do to ameliorate these effects? | | |

| **Bad breath** | Yes ☐ | No ☐ |
|---|---|---|
| Do you remember on which date (or thereabout) this occurred? | | |
| Can you give further details of what happened? | | |
| Can you remember how long this lasted? | | |
| How severe would you say these effects were? | | |
| What did you do to ameliorate these effects? | | |

| **General weakness** | Yes ☐ | No ☐ |
|---|---|---|
| Do you remember on which date (or thereabout) this occurred? | | |
| Can you give further details of what happened? | | |
| Can you remember how long this lasted? | | |
| How severe would you say these effects were? | | |

*For the researcher only:*

| | | |
|---|---|---|
| **Reported as adverse event? (Y/N)** | Yes ☐ | No ☐ |
| **AE reference no and date** | *Please note that each occurrence reported above will create a separate AE record.* | |

Semi-structured questionnaire on experience with dietary intervention

**CALIBER (Carbohydrates, lipids and biomarkers of traditional and emerging cardiometabolic risk factors) Phase 1: A pilot study in normal-weight and overweight adults**

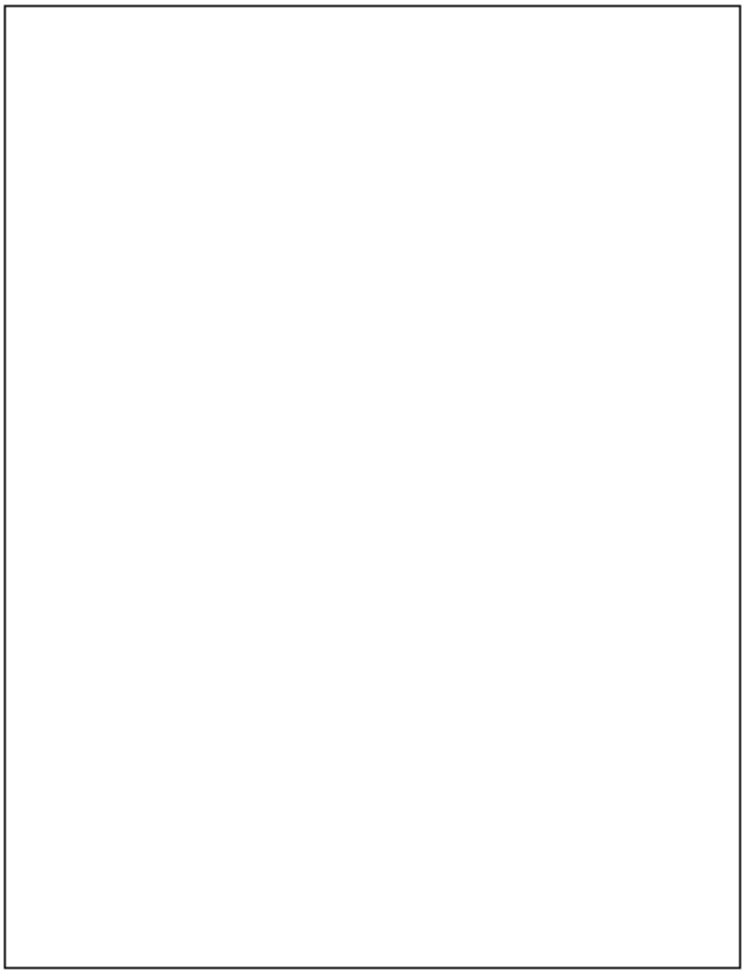
Participant ID Date

Allocated diet:

**Over the past 8 weeks how easy or difficult was it for you overall to follow the diet allocated to you?**

| | |
|---|---|
| **Very easy** | |
| **Somewhat easy** | |
| **Not sure** | |
| **Somewhat difficult** | |
| **Very difficult** | |

**Could you please explain your answer?**



Which aspects of the diet allocated to you did you enjoy?

Which aspects of the diet allocated to you did you not enjoy?

Over the past 8 weeks did you or the person responsible for shopping and cooking in your household use the recipes and example meal plans provided to you?

| Yes | No | Not sure |
|---|---|---|

If no, can you please explain why you/they did not use the recipes and example meal plans provided?

Over the past 8 weeks did you or the person responsible for shopping and cooking in your household use the Facebook group for advice and support?

| Yes | No | Not sure |
|---|---|---|

If no, can you please explain why you/they did not use the Facebook group for advice and support?

If you or the person responsible for shopping and cooking in your household did use the Facebook Group what did you/they like about it?

If you or the person responsible for shopping and cooking in your household did use the Facebook Group what did you/they not like about it?

Over the past 8 weeks compared to your usual diet how did you or the person responsible for shopping and cooking in your household find shopping for the ingredients of the diet allocated to you?

| | |
|---|---|
| A lot easier | |
| Somewhat easier | |
| About the same | |
| Somewhat harder | |
| A lot harder | |
| Not sure | |

Could you please explain your answer?

| |
|---|
| |

Over the past 8 weeks compared to your usual diet how did you or the person responsible for shopping and cooking in your household find cooking according to the rules of the diet allocated to you?

| | |
|---|---|
| A lot easier | |
| Somewhat easier | |
| About the same | |
| Somewhat harder | |
| A lot harder | |
| Not sure | |

Could you please explain your answer?

| |
|---|
| |

Over the past 8 weeks how expensive compared to your usual diet did you or the person responsible for shopping and cooking in your household find the diet allocated to you?

| | |
|---|---|
| A lot cheaper | |
| Somewhat cheaper | |
| About the same | |
| Somewhat more expensive | |

| | |
|---|---|
| A lot more expensive | |
| Not sure | |

Could you please explain your answer?

| |
|---|
| |

Over the past 8 weeks how did you find it to follow the diet allocated to you in social situations (e.g. eating out, going out, dinner parties, occasions, etc.)?

| | |
|---|---|
| A lot easier | |
| Somewhat easier | |
| About the same | |
| Somewhat harder | |
| A lot harder | |
| Not sure | |

Could you please explain your answer?

| |
|---|
| |

Over the past 8 weeks how did you find it to follow the diet allocated to you in your home environment (e.g. eating as a family/couple/etc)?

| | |
|---|---|
| A lot easier | |
| Somewhat easier | |
| About the same | |
| Somewhat harder | |
| A lot harder | |
| Not sure | |

Could you please explain your answer?

| |
|---|
| |

Over the past 8 weeks following the diet allocated to you how have you felt with regards to happiness compared to following your usual diet?

| | |
|---|---|
| A lot happier | |
| Somewhat happier | |
| About the same | |
| Somewhat unhappier | |
| A lot unhappier | |
| Not sure | |

Could you please explain your answer?

| |
|---|
| |

Over the past 8 weeks following the diet allocated to you how have you felt with regards to health compared to your following your usual diet?

| | |
|---|---|
| A lot healthier | |
| Somewhat healthier | |
| About the same | |
| Somewhat unhealthier | |
| A lot un healthier | |
| Not sure | |

Could you please explain your answer?

| |
|---|
| |

Over the past 8 weeks following the diet allocated to you how have you felt with regards to alertness and cognition compared to following your usual diet?

| | |
|---|---|
| A lot more alert | |
| Somewhat more alert | |
| About the same | |
| Somewhat less alert | |
| A lot less alert | |
| Not sure | |

Over the past 8 weeks following the diet allocated to you do you feel that your sleep and sleeping patterns have improved compared to following your usual diet?

| | |
|---|---|
| I slept a lot better | |
| I slept somewhat better | |
| About the same | |
| I slept somewhat worse | |
| I slept a lot worse | |
| Not sure | |

Could you please explain your answer?

| |
|---|
| |

Over the past 8 weeks following the diet allocated to you do you feel that energy levels have changed compared to following your usual diet?

| | |
|---|---|
| I feel a lot more energetic | |
| I feel somewhat energetic | |
| About the same | |
| I feel somewhat less energetic | |
| I feel a lot less energetic | |
| Not sure | |

Could you please explain your answer?

| |
|---|
| |

Given your experience with your allocated diet if this showed to improve your cardiometabolic health would you feel that you were able to continue with it in the future?

| Yes | No | Not sure |
|---|---|---|

Could you please explain your answer?

| |
|---|
| |

Over the past 8 weeks do you feel that you were given sufficient support to help you follow you allocated diet?

| Yes | No | Not sure |
|---|---|---|

Could you please explain your answer?

| |
|---|
| |

Over the past 8 weeks did you find it easy to attend the appointments at IM Marsh campus?

| Yes | No | Not sure |
|---|---|---|

Could you please explain your answer?

Are there any other comments that you would like to make?

# Low-carb Group


**CALIBER study Merseyside**

Liverpool John Moores University

School of Sport Studies, Leisure and Nutrition

Tanja Harrison BSc (Hons) ANutr AFHEA

SEDA

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M 07970 858 594

## Contents

## Quick guide

For the next eight weeks you have been allocated to the low-carb group of the CALIBER study, which means that you will be asked to follow a diet containing a maximum of 50 grams of carbohydrates per day.

We will undertake a variety of assessments with you when you come to see us in our laboratory at IM Marsh campus on three occasions, including blood samples, body composition, dietary intake and impact of this diet on food cravings and cognition. We will also ask you to wear a wrist-worn physical activity monitor on three occasions.

These guidance notes will give you further information on which types of food to eat and not to eat on a low-carb diet and how to read food labels and become aware of the nutritional content of out-of-home cooked meals to support your efforts to stick to this eating plan.

In this booklet, you will also find the names and contact details of the people involved in this research.

## Introduction

Hello and welcome to the CALIBER study! Nice to have you on board.

This booklet has been designed to be your companion over the next eight weeks whilst you are part of our cohort and to support you to consume a healthy diet whether you are cooking at home, buying ready-made meals or eating out. There are certain rules that can be applied to all of these situations.

We will also give you information on the purpose of our study, what to expect during your visits to our laboratories and once the study has finished and we have analysed the results. This means that not only will you help an important research cause but you can also find out how you and your body did over the course of these eight weeks and how your body composition, blood profile, food cravings and cognition might have been affected.

We hope that you will find your time on the study interesting, inspiring, motivating and delicious.

## What do we want to find out?

Maybe you have followed the news over these past couple of years and noticed that there is a lot of controversy and discussion about what makes a diet healthy. The debate has been particularly heated around the issue of carbohydrates. Whilst many public health officials have argued that the vast majority of the population does not follow the dietary UK guidelines (which can be classed as high-carbohydrate, moderate-fat) and that this is the root cause of the UK's problem with ill-health, obesity and diseases such as heart disease and type 2 diabetes, others claim that these guidelines have caused these problems to begin with. The latter group advocates to reduce the amounts of carbohydrates we consume as a nation and for the guidelines to be re-written.

A third group suggests that it is far more complicated than this and that how we react to carbohydrates is actually far more personal and not one-size-fits-all, but some people might be better off on low-carb diets whilst others fare better on high-carb diets in terms of cardiometabolic health.

This is where our study comes in, CALIBER – Carbohydrates, lipids and biomarkers of traditional and emerging cardiometabolic risk factors. We want to compare the effects of consuming either a high-carb or a low-carb diet on these risk factors. The reason why we have asked you to join us is because you showed some slightly elevated risk markers for these illnesses, albeit still at a stage where these should be easy to control and improve through a healthy diet, be this in form of low-carb, high-fat or high-carb, moderate-fat.

The discussion surrounding carbohydrates does not simply stop at their potential impact on risk markers that we find in our blood. There is also some discussion whether carbohydrates or fats lead to an increase in waist circumference and the development of fat deposits in our bodies, whether carbohydrates or fats are the root cause of food cravings that we might experience and whether one or the other somehow helps us to move about more or makes us more sluggish.

As scientists and nutritionists we are naturally curious to find out what might be going on. So thank you for joining us!

## What to expect during you visits to our lab

There will be three appointments where we will ask you to come to IM Marsh campus (L17 6BD) for more thorough assessments, one right at the start when you will commence to eat according to the guidelines given to you, one after about 4 weeks and the final visit 8 weeks after your first one.

Each visit is expected to last between 60 and 90 minutes and will entail

**A venous blood sample**

We take about 8 teaspoons of blood. – Please note that you will have to have fasted for at least 12 hours prior to your appointment as otherwise your blood sugar and your triglycerides might be far higher than normal – painting a wrong picture of how the eating plan is working for you. Just as you had to do before coming in for your initial finger prick appointment you will also have to restrain from drinking alcohol or undertaking any strenuous exercise the night before. Again both can have an impact on your blood profile! We will analyse this blood sample at the end of the study to see how any risk factors for heart disease and type 2 diabetes might have changed over the course of eight weeks.

**Assessing your body composition**

This will be done in two different ways. Firstly, we will use a tape measure to measure your waist circumference, hip circumference, thigh circumference, calf circumference and neck circumference as these are all sites on the human body that can give us clues about the overall distribution of body fat. – **Please ensure that you bring a pair of shorts with you** to these visits as we will ask you to change into these before we take these measurements. If you prefer for a team member of the same sex to take these, please do let us know so that we can ensure that this can be facilitated.

Secondly, we will ask you to step onto sophisticated body composition scales (far bigger than the common bathroom ones) and measure your lean body mass, your body fat mass and the amount of fat surrounding your organs.

**Taking your blood pressure**

As blood pressure has been found to be an important factor in cardiometabolic health, we will assess your blood pressure every time you come to see us in our labs. Following standard protocol, we will take your blood pressure three times at each appointment and calculate the average of these three.

**Going through a couple of brief questionnaires with you and conducting one final interview**

During your second and your final visit we will go through a check list to see which types of fibre-containing foods you have consumed over the previous four weeks. We will also conduct a brief interview to check whether you have experienced any so-called adverse events over the past 4 weeks whilst you were eating according to the low-carb rules.

We will also ask you to bring the container containing your multivitamin and mineral supplement with you containing any remaining pills. During your second lab visit we will provide you will a further supply of supplements for the final four weeks of the study.

During your final visit we will also ask to stay with us for a little longer to conduct a brief interview with you asking you about your experiences with the diet allocated to you.

**Prior to your lab appointments – recording of physical activity**

On three occasions (just before your first, second and final visit to our lab) we will ask you to wear a physical activity monitor that looks like a digital watch and has to be worn on the wrist (just like a 'Fit bit') of your non-dominant arm. That means if you are right-handed you will have to wear this on your left wrist.

We will give you this device at least 8 days before your visits to our labs and will ask you to start wearing it for 7 days and at least 10 hours per day commencing on the morning after it has been handed to you and finishing the night before your lab appointment. During this time you will have to complete a wear-time diary on a daily basis, in which you will briefly record the times you are putting the monitor on in the morning, the times you are taking it off at night and any time during the time when you need to remove and put it back on, for example when you are taking a shower or when you are going swimming.

You will need to bring the monitor and the wear time diary with you on the morning of your lab appointment where the research team can collect it from you.

**Prior to your lab appointments – food diaries and questionnaires**

When you are given your physical activity devices we will also give you a template of a four-day food diary, which you will need to complete for four days before your lab appointment and bring with you on the morning.

We will also give you a number of brief questionnaires, which will assess your cognition and your food cravings over the previous four-week period. These will not take long to complete. Please also bring these questionnaires with you to your lab appointment and pass them on to our research team.

## Your involvement in the study – Flow chart low-carb group

## Your appointment schedule

| Physical activity monitor collection//delivery | First lab visit | Physical activity monitor collection/ /delivery | Second lab visit | Physical activity monitor collection/ /delivery | Third lab visit |
|---|---|---|---|---|---|
| | | | | | |

All lab visits will take place at LJMU IM Marsh campus in Mossley Hill (L17 6BD).

## Your contacts

Your main contact is Tanja Harrison who is also a registered associate nutritionist with the Association for Nutrition.

M 07970 858 594

E T.harrison@2015.ljmu.ac.uk

If you have any queries or concerns throughout the study, please do not hesitate to contact Tanja.

Other researchers on the team that you will meet on a regular basis

Deaglan McCullough

E d.mcullough@2015.ljmu.ac.uk

Dr Ian Davies (A registered nutritionist, focus on nutrition science)

E i.g.davies@ljmu.ac.uk

Dr Katie Lane (A registered nutritionist, focus on food)

E k.e.lane@ljmu.ac.uk

Dr Kevin Enright

E k.j.enright@ljmu.ac.uk

This study was approved by LJMU's Research Ethics Committee on 16 December 2016 (Ref. 16/ELS/029). If you any concerns regarding your involvement in this research, please discuss these with the researcher in the first instance. If you wish to make a complaint, please contact researchethics@ljmu.ac.uk and your communication will be re-directed to an independent person as appropriate.

## Further information throughout the study

You can also find all the materials in a private Facebook Group, which you can request to join should you wish to do so at https://www.facebook.com/groups/lowcarbCALIBER/

We will also post regular updates here. However, should you not wish to join all the links and information will be provided to you via email or in hard copy.

## Following a low-carb diet – What does that actually mean?

This guideline has been designed to help you follow a low-carbohydrate, high-fat diet over the next eight weeks whilst you are part of our cohort.

The diet that has been assigned to you means that you will eat only small amounts of carbohydrates per day and a higher proportion of fat. Whilst you are taking part in study we ask you to consume a minimum of 30 grams and a maximum of 50 grams of carbohydrates per day.

On a usually consumed Western diet the human body uses the glucose derived from carbohydrates for nearly all its energy needs. However, when carbohydrate intake is restricted the body learns to adapt very quickly and uses dietary and body fat instead. This happens through a process called "ketosis", those fatty acids consumed with our foods and those stored within our body fat cells are broken down in order to produce glycerol and ketone bodies. On very low-carb diets these products will provide the energy required by the human body for normal functioning.

In terms of your diet this means that you will instead eat more fat which will come from a variety of sources.

Carbohydrates come from a range of foods, including potatoes, rice, pasta, bread, fruit and vegetables and to a lesser extend dairy products (in the form of milk sugar, called lactose). This means that there will be restrictions as to which and how much of these foods you will be able to consume.

## Potential beneficial effects of a low-carb diet

There is a reason why a growing number of nutrition and health professionals are in favour of a low-carb, high-fat diet.

- You should feel less hungry, especially between meals
- This means that you might eat less than you usually do, leading to a lower calorie intake and consequential potential weight loss
- Your body composition might change meaning less fat mass
- Your insulin levels should decrease as your body needs to produce less insulin to maintain blood sugar levels after a meal
- You will have better blood sugar control, which means that your body needs to produce less insulin to ferry the glucose in your blood into your muscles and you fat depots
- Your cravings for sugary and fatty foods should reduce which means that you will probably eat less of these foods.
- You might feel less bloated

## Potential adverse effects of a low-carb diet

However, as we have already told you when you signed up – remember that disclaimer? – some people might have a bit of a harder time adjusting to the new diet. Especially during the first few days on a very low-carb diet you might experience

- Headaches - Drink plenty of fluids.
- Lethargy, weakness - If this is the case try and take it easy for a few days.
- Constipation - This is due to the potentially lower fibre intake. You can address this by increasing your fibre intake and drinking plenty of fluids. Please see the list of fibre-rich permitted foods on page 15.
- Muscle cramps -This is due to your kidneys excreting more sodium which can also impact on your potassium balance. Sodium and potassium are minerals which are used by your body as electrolytes, meaning they regulate the fluid balance in our bodies and stimulate our muscles and nerves. You can deal with this by continuing to take your daily multivitamin and mineral supplement provided by us and by drinking cups of bouillon or home-made bone broth (see recipe). You can also add half a teaspoon of salt to one litre of water and drink this throughout the day. In addition, consume plenty of magnesium and potassium-rich foods from the list provided.
- Brain fog – As your brain has used carbs so far to do all its hard work the initial switch from one fuel (carbs) to another (fats) might take a few days. If you can, try and take it easy for a few days and drink plenty of fluids.
- Nausea, anxiety and palpitations - This might also be due to your electrolyte deficiency (Magnesium and potassium).
- Poor sleep quality – After a while, however, a lot of people report a better quality of their sleep
- Bad breath – A sugar-free mint might help. Bad breath is a sign of your body switching from using carbs to fats (ketones) as fuel source. It will pass!
- Consumption of potentially fewer vitamins and minerals – This is why we are giving you your daily multi-vitamin and mineral supplement.

For these reasons we are actually advising you to start the diet on a less busy day of your week.

## How to take your multivitamin supplement

During your first lab visit when you give your first venous blood sample we will also provide you with a multivitamin and mineral supplement, which you will need to take on a daily basis. The container includes 30 pills, which should be sufficient until your second lab appointment where you will receive the supplements for the following 4 weeks.

Please bring the pill container with you to each lab appointment, as we will need to count the number of pills left in the container. We are doing this as the number of pills taken will be taken into account when we analyse the data. It will also help us to explain why some side effects might have occurred – for example if you forgot very often to take your supplement.

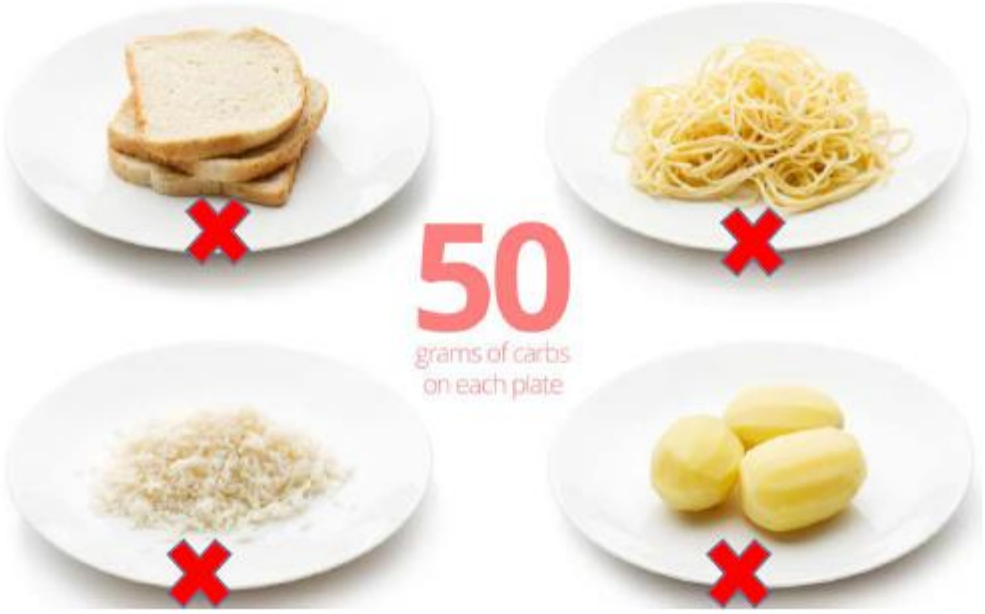
Please do remember to take it on a daily basis. Preferably at mealtimes as some of the vitamins can only be absorbed by our bodies when consumed at the same time as fat. Having said this, the other vitamins will also be more readily available to your body when other nutrients have to be digested with them.

## Portion sizes for carbohydrates

So, what do 50 grams of carbs look like?

As there are foods that are naturally high in carbs and those that are naturally low in carbs it can be confusing to decide how much you could eat of these foods.

Below you can see examples of what 50 grams of carbs might look like and how easy it would be to exceed your daily allowance.




*(Source: The Dietdoctor, 2017)*


*(Source: The Dietdoctor, 2017)*

Please remember that the foods shown above are on the plates in isolation. Add to these the rest of the foods that you will consume throughout the day and all those carbs soon add up! The foods on the first picture will therefore be found on the list of food items that you will not be allowed to consume over the next eight weeks (see below).

## What is fibre and why are we supposed to eat it?

Carbohydrates in our diet come from different sources, with some of them being more readily (if at all) absorbed by our bodies and used for energy to keep us going during the day. When nutritionists and other health professionals talk about carbohydrates and a certain type of carbohydrate diet (in this case "low carbohydrate diet") we mean carbohydrates that are actually available to our bodies. Only these types of carbohydrates are actually being counted when making recommendations for carbohydrate intake. The other type of carbohydrates, which are generally not available, are classed as dietary fibre (see below). There are also different subcategories of these, with some supplying our bodies with small amounts of energy, but these can be disregarded in the context of the foods that we consume every single day as part of a healthy balanced diet.

In the UK it is recommended that everyone in the UK above the age of 14 should aim to consume at least 30 grams of fibre per day. At the moment the majority of the population is not meeting these recommendations. Research has shown that sufficient fibre intake can help prevent heart disease, some cancers and diabetes. Fibre can also aid to improve your digestion and make you feel fuller for longer meaning you eat less and less often (serial snackers beware). Fibre can be found in a number of fruits, vegetables and nuts and seeds, i.e. plant-based foods.

Foods that contain 6 or more grams of fibre per 100g are classed as high-fibre foods, whereas those containing at least 3 grams of fibre per 100g are considered to be 'fibre-rich'.

Please ensure that if you think that your diet has been lacking in fibre so far to increase the amounts that you are consuming gradually over a couple of weeks and to make sure that you drink plenty of fluids. Otherwise you might have to deal with bloating and constipation as side effects.

## Carbohydrate and fibre content of fruit and vegetables permitted on a low-carb diet

| Food name | Amount available (digestible) carbohydrates in g (per 100g) | Amount fibre in g (per 100g) | Amount available (digestible) carbohydrates in g (per 80g portion) | Amount fibre in g (per 80g portion) |
|---|---|---|---|---|
| Asparagus, boiled | 1.4 | 1.4 | 1.12 | 1.12 |
| Aubergines, fried | 2.8 | 2.3 | 2.24 | 1.84 |
| Avocado, average | 1.9 | 3.4 | 1.52 | 2.72 |
| Beans, green, boiled | 4.0 | 2.5 | 3.2 | 2.0 |
| Beans, soy/edamame | 5.1 | 6.1 | 4.08 | 4.88 |
| Broccoli, boiled | 2.8 | 2.3 | 2.24 | 1.84 |
| Broccoli, Purple sprouting, boiled | 1.3 | 2.3 | 1.04 | 1.84 |
| Brussel sprouts, boiled | 3.5 | 3.1 | 2.8 | 2.48 |
| Cabbage, green cooked | 2.3 | 2.6 | 1.84 | 2.08 |
| Cabbage, red cooked | 2.3 | 2.0 | 1.84 | 1.6 |
| Cabbage, white cooked | 3.2 | 1.4 | 2.56 | 1.12 |
| Cabbage, spring greens, cooked | 1.6 | 2.6 | 1.28 | 2.08 |
| Carrots, young, boiled | 2.3 | 1.84 | 4.4 | 3.52 |
| Cauliflower, cooked | 3.5 | 1.6 | 2.8 | 1.28 |
| Celery, raw | 0.9 | 1.1 | 0.72 | 0.88 |
| Coconut, desiccated | 6.4 | 13.7 | 5.12 | 10.96 |
| Coconut, fresh | 3.7 | 7.3 | 2.96 | 5.84 |
| Courgette, boiled | 2.0 | 1.2 | 1.6 | 0.96 |
| Cucumber, raw | 1.2 | 0.7 | 0.96 | 0.56 |
| Fennel, Florence, boiled | 1.5 | 2.3 | 1.2 | 1.84 |
| Kale, curly, boiled | 1.0 | 2.8 | 0.8 | 2.24 |
| Kohlrabi, boiled | 3.1 | 1.9 | 2.48 | 1.52 |

| Food name | Amount available (digestible) carbohydrates in g (per 100g) | Amount fibre in g (per 100g) | Amount available (digestible) carbohydrates in g (per 80g portion) | Amount fibre in g (per 80g portion) |
|---|---|---|---|---|
| Leeks, boiled | 2.6 | 1.7 | 2.08 | 1.36 |
| Lettuce, cos | 1.19 | 2.1 | 0.95 | 1.68 |
| Lettuce, iceberg | 1.77 | 1.2 | 1.42 | 0.96 |
| Lettuce, romaine | 1.19 | 2.1 | 0.95 | 1.68 |
| Mushrooms, oyster, raw | 3.79 | 2.3 | 3.03 | 1.84 |
| Mushrooms, Portobello, grilled | 2.24 | 2.2 | 1.79 | 1.76 |
| Mushrooms, white, boiled | 0.1 | 2.1 | 0 | 1.68 |
| Olives, green in brine, drained | 0.0 | 2.9 | 0 | 2.32 |
| Onions, fried (based on 2 table spoons, chopped) | 11.2 | 1.5 | 3.36 | 0.45 |
| Pepper, green, boiled | 2.6 | 1.8 | 2.08 | 1.44 |
| Pepper, red, boiled | 3.4 | 0.8 | 2.72 | 0.64 |
| Pepper, yellow, boiled | 5.3 | 0.8 | 4.24 | 0.64 |
| Pumpkin, boiled | 1.9 | 1.1 | 1.52 | 0.88 |
| Radishes, raw | 1.9 | 0.9 | 1.52 | 0.72 |
| Raspberries, red | 4.6 | 2.5 | 3.68 | 2.0 |
| Rocket | 0.0 | 1.3 | 0.0 | 1.04 |
| Spinach, baby, raw | 0.2 | 1.2 | 0.16 | 0.96 |
| Spinach, frozen, boiled | 0.5 | 2.1 | 0.4 | 1.68 |
| Spring onion, raw | 3.0 | 1.5 | 2.4 | 1.2 |
| Strawberries | 6.1 | 1.0 | 4.88 | 0.8 |
| Tomato, raw | 3.0 | 1.0 | 2.4 | 0.8 |
| Turnip, boiled | 2.0 | 1.9 | 1.6 | 1.52 |
| Watercress | 0.4 | 1.5 | 0.32 | 1.2 |

### Carbohydrate and fibre content of nuts and seeds

A small handful (or about 30g) of nuts or seeds counts as a portion. Below some guidelines of how much fibre you would get from different types of nuts and seeds.

| Food name | Amount available (digestible) carbohydrates in g (per 100g) | Amount fibre in g (per 100g) | Amount available (digestible) carbohydrates in g (per 30g portion) | Amount fibre in g (per 30g portion) |
|---|---|---|---|---|
| Almonds, raw | 2.5 | 2.7 | 0.75 | 0.81 |
| Brazil nuts | 3.1 | 4.3 | 0.93 | 1.29 |
| Chia seeds | 6 | 38 | 1.8 | 11.4 |
| Flaxseeds (Linseeds) | 2 | 27 | 0.6 | 8.1 |
| Hazelnuts | 6.0 | 6.5 | 1.8 | 1.95 |
| Pecan nuts | 5.8 | 4.7 | 1.74 | 1.41 |
| Pumpkin seeds | 5.71 | 6.0 | 1.71 | 1.8 |
| Walnuts | 3.3 | 3.5 | 0.99 | 1.05 |

## Permitted foods on a low-carb diet

### Dietary fat – the star of the show? Following a high-fat diet



Cutting down on carbs to the extent that is necessary on a low-carb diet means that your energy needs to come from somewhere. Earlier on we already mentioned that fat can also be broken down into products (ketones) that supply our bodies with energy after a period of adaptation.

Fat is also important role in our diet as it helps to absorb some vitamins that would otherwise simply go right through us. It also plays an important role in building the membranes of our cells. The key lies in the quality of the fats consumed with priority given to unsaturated fats, such as olive oil and other vegetable oils, avocados, nuts and oily fish. One type of unsaturated fat, which is essential to human health, are omega-3 fatty acids. These can be found both in animal and plant sources. Oily fish (see below) is an important source of omega-3 fatty acids as these are of the highest quality. However, it is possible for the body to convert the omega-3 fatty acids found in plants foods, such as nuts and seeds and their products, into the same end products found in oily fish. Coconut oil is also a fat recommended on a low-carb, high-fat diet and it is thought to be very healthy, despite a high proportion of saturated fats in it. Some people love to use it for everything, including frying their eggs. However, a small word of caution when it comes to taste as this is very distinct in coconut oil. For some individuals the egg-frying

method might be a bit too much for this reason. In other recipes, however, such as curries, soups and other dishes with lots of other ingredients, the taste of coconut oil complements and enhances the dish instead of tasting too overpowering.

Saturated fats have received a lot of bad press over the past few decades and whilst it is important to prioritise the fatty acids introduced above (mono- and polyunsaturated) a low-carb, high-fat diet doesn't place the same restrictions on saturated fats that you might have been used to so far. A lot of foods that contain a fairly high amount of saturated fats, such as meat, butter and cheese are actually encouraged to be consumed. You therefore don't need to worry about removing the visible fat from beef and pork steaks and other meats or about removing the skin from chicken. This will be one way of ensuring that you will achieve the amount of fat you will need to consume to replace the carbohydrates that you will omit from your diet. In addition, the marbling on cuts of meat will enhance the flavour whilst cooking it and also ensure that the meat stays moist during the cooking process.

One thing to avoid on a low-carb, high-fat diet are margarines as these are not a natural fat but have been manufactured and hardened from vegetable oils – a highly processed food. You should also not use vegetable oils that are high in omega-6 (as opposed to omega-3) fatty acids, such as sunflower oil, corn oil, soybean oil and cotton seed oil. Vegetable oils that should be consumed instead are coconut oil, olive oil and rapeseed oil

### *Dairy*

Dairy products are both a source of fats and also proteins. Consuming a low-carb, high-fat diet means that you should not shy away from using full-fat versions of the product. Think butter, cream and jersey milk or at least full-fat milk. Stay away from low-fat yoghurts and also avoid fruit yoghurts as these contain too much sugar. Dairy is a good source of calcium and should therefore be included in our diet on a daily basis. However, you will also have to careful with the amount of milk that you are consuming as the milk sugar also contains glucose which will be absorbed by our bodies. A bit of milk (full-fat) in your tea and coffee is fine. However, a milkshake or hot chocolate should only be an occasional treat and then you still have to be mindful of the other ingredients.

Dairy products good to consume on a low-carb diet include full-fat versions of cheese, cream, cream cheese, crème fraiche, sour cream, quark and yoghurt.

## Proteins to help you build and maintain that temple which is your body

Proteins are important in our diet as they have the vital functions of growth, maintenance and repair. They are also vital to help our immune system function properly. Proteins are composed of compounds called *amino acids* of which there are 20 that play a role in the human body. 8 of these amino acids are classed as essential because unlike the other 12 our bodies cannot produce these themselves, which means that they need to be obtained from our diets. This happens by consuming protein foods.

Whilst protein malnutrition can have disastrous consequences in reality the vast majority of people in the UK consume more than they actually need to.

The average UK male aged 15 to 64 should consume about 55 grams per day, the average UK female in this age group 45 grams per day. In order to achieve these recommendations we should eat two to three portions of protein each day. There are different types of protein sources permitted on a low-carb diet, including:

### Animal-based proteins

Animal-based proteins contain all of the essential amino acids. This is because the animals have done the work for us eating a variety of food sources with different amino acid profiles and combing these in their muscles. The same is true for eggs where the egg yolk and the egg white have the role of sustaining the developing chick.

#### *Meat*

Meat is permitted on a low-carb diet as it contains virtually no carbohydrates. However, you should not eat more than 70g of processed meat per day, which includes sausages, bacon, cured meats (for example salami, chorizo) and reformed meats products (for example sliced packaged ham). Even on a low-carb diet consuming these foods in excess is not healthy as these not only tend to contain a number of additives but are also high in salt. Unfortunately, depending on the type of processed meat, the manufacturing process and the reputability of the manufacturer this might also mean that you might be consuming a low-quality product consisting of offcuts that will only be saleable if disguised in this form.

You should also be careful with deli products such as pates and hams (breaded is a no-no!) as these might contain hidden sugars, so it is best to check first before eating or even buying them.

#### *Fish and seafood (Shellfish)*

You should eat at least two portions of fish per week, one white, one oily. One portion is 140 grams which is about the size of a cheque book. The reason we recommend oily fish because these are an excellent source of omega-3 essential fatty acids, which we have mentioned earlier on. Fish also supply us with a number of vitamins and minerals. Remember that two portions per week is the minimum.

Please remember that you will not be able to eat battered or breaded fish whilst on low-carb!

**White fish**

| | | | | | |
|---|---|---|---|---|---|
| Basra | Cod, | Coley | Dab | Flounder | Gurnard |
| Hake | Haddock | Plaice | Pollock | Red mullet | Tilapia |

Some white fish should be eaten no more than once a week due to potentially high levels of pollutants contained in their flesh. These are seabream, seabass, halibut and turbot.

**Oily fish**

| | | | | | |
|---|---|---|---|---|---|
| Anchovies | Carp | Herring | Kippers | Mackerel | Pilchard |

| | | | | | |
|---|---|---|---|---|---|
| Salmon | Sardines | Sprats | Trout | Tuna (fresh or frozen) | Whitebait |

Swordfish should not be eaten more than once a week due to potentially high levels of pollutants. It should be avoided by children, pregnant women and those wanting to become pregnant.

**Shellfish**

| | | | |
|---|---|---|---|
| Cockles | Crab | Langoustines | Mussels (and clams and winkles) |
| Oysters | Prawns | Scallops | Squid |

It is important to note that processed, canned fish like tuna do no longer count as your 'oily' portion as the manufacturing process has taken all the omega-3 fatty acids out of them. However, the good news is – that tuna salad for your lunch still counts towards your portion of white fish.

One thing to bear in mind is choosing where possible sustainable sources of fish as recommended by the Marine Stewardship Council (MSC) in their *Good Fish Guide*. The MSC also run an accreditation scheme, so look out for certified products carrying the following logo:

Further information on the MSC and a regularly updated list of sustainable types of fish can be found at www.msc.org.

*Eggs*

Eggs are real powerhouses of nutrition and contain all the essential amino acids that humans need in their diet. One medium egg provides about 6.4grams of protein with the egg yolk containing higher concentrations of amino acids. However, as the egg white is larger than the egg yolk the majority of protein supplied when eating an egg will actually come from the egg white.

For animal welfare reasons we would recommend that you avoid buying the eggs from caged hens. However, the choice is absolutely yours.

A word on dairy products

Dairy products, such as milk, cheese, cream, sour cream and yoghurt, are also good sources of protein. However, as they also contain a fair amount of fat, we have included them in the fat section of this guide.

Plant-based proteins

Nuts and seeds are a great source of proteins and are also great sources of unsaturated fats, included important omega-3s.

Other plant-based protein sources include tofu, bean curd, mycoprotein (such as Quorn™) and soya-based meat replacement products, such as supermarkets' own brands. Please remember that the latter two are processed foods so it is always worth checking the label to ensure that there are not too many additives and flavour enhancers, salt and sugar found in

these products. If this is the case, you should either eat these products sparingly to ensure that you do not end up eating lots of hidden sugars by accident!

Beans and pulses, including lentils, although good sources of protein, are generally not recommended on a low-carb diet with the exception of green beans and soya beans as these don't contain as many carbs but are good sources of fibre.

### Portion sizes for protein

**Non-dairy**

| | |
|---|---|
| Eggs | 2 medium-sized |
| Fish | 140g (size of a cheque book) or 3 fish fingers |
| Meat, cooked | 80g (size of a deck of cards or 3 slices) |
| Meat-replacement products | 120g (for example two sausages or about the size of a snooker ball) |
| Nut butters, such as peanut butter | 2 tablespoons (about the size of a golf ball) |
| Nuts and seeds | 40g (1 small handful) |

### Vegetables

Vegetables growing above ground, for example artichokes, asparagus, aubergines, avocado, bok choi, broccoli, Brussel sprouts, cabbage, cauliflower, courgettes, cucumber, kale, lettuce, mushrooms (careful with shiitake mushrooms however, as they contain a lot more carbohydrates that other varieties!), olives, onions, peppers, spinach and other leafy greens, and tomatoes (Technically a fruit – we know ;-))

You have to be very careful with eating sweetcorn, corn on the cob and peas as these are higher in carbohydrates than other vegetables growing above ground and should only be eaten in moderation.

### Fruit

As fruits contain a lot of carbs they should be eaten in moderation, only about 1 piece of fruit per day. Bananas and grapes are the worst offenders in a low-carb diet context. Berries can be eaten a bit more regularly but you should also be careful with these.

There are actually several reasons why eating vegetables and (some) fruits is good for you. Firstly, they contain a considerable amount of carbs in the form of fibre – the good stuff. Secondly, the different colours express the presence of different types of nutrients that are really good for us and are thought to prevent heart disease, diabetes and cancers. These are namely anti-oxidants and polyphenols. The different colours of the fruit and vegetables available for us mean that these contain different types of these nutrients – all-round protection.

If you feel slightly adverse to anything green and orange on your plate (one too many roast dinners with boiled-to-death cabbage) there are ways of incorporating these to help you feel less annoyed by their presence and to gradually get used to them. You could for example add some finely chopped carrots and vegetables to a tomato sauce with your fish or chicken.

Alternatively, you could make a mushroom pâté to create a delicious snack eaten with vegetable sticks.

## Magnesium-rich foods

Magnesium plays an important role in our diet and obtaining it from a carefully planned low-carb diet is possible. However, there are some foods that will make it more likely that you will meet your daily requirements. These are artichokes, bone broth (we will give you a recipe for this), fish (highest are fresh tuna and halibut), nuts, spinach and other leafy greens.

## Potassium-rich foods

Like magnesium, potassium is also important in a healthy, balanced diet and there are some foods that should be preferred to others in order to achieve your recommended intake. These are avocados, bone broth (again recipe to follow), cooked greens, cooked mushrooms, fish, meat and tomatoes.

## Food no-no's on a low-carb diet

Sugar in any of its natural forms – This includes table sugar, brown sugar, demerara etc; honey, syrups in any form, molasses. Be aware of hidden sugars in food products and ready-meals. Later on in this guide we will show you how to read labels and identify these silent assassins that might jeopardise all your efforts.

If you can't do without sweetness for example in your tea or coffee there are some alternatives (in form of natural low-carb sweeteners and sugar alcohols) on the market that you can use sparingly. These are

- Stevia – a plant extract which is 200 to 300 times sweeter than sugar and has been used as a sweetener for many years in Asia and South America. Stevia is sold in UK supermarkets as Stevia sweetener and can also be found under the brand name "Truvia". However, be careful not to pick up a stevia-sugar blend by accident!
- Erythritol – a so-called sugar alcohol, which has about 70% of the sweetness of sugar but does not get broken down by our bodies (unlike sugar). Erythritol can be ordered online.

However, if you feel or experience that the sweet taste of these sweeteners induces cravings for sweet foods and carbs in general (which can happen in some cases) we would advise you to take the plunge and have your tea and coffee unsweetened.

As you will have probably expected chocolate is on the list of foods not to eat over the next 8 weeks. If you are really desperate you can have that odd piece of small (and we mean small!) piece of 70% or above dark chocolate, which contains about 3.5 grams of carbs per square. If you feel that you have to eat more than this small piece once you have started it is best to not eat it at all!

## So what should you eat in a day if you are following a low-carb, high-fat diet

Following a low-carb, high-fat diet means that you can eat lots of permitted vegetables (preferably green as they are lower in carbs – but do incorporate some colour as well) and as many sources of high-quality fats as you like – avocado, butter, nuts, seeds and anything from the recommended dairy range. Fat has more calories per gram than carbs or proteins and will help you to feel full and satisfied quicker. Don't forget that if you have eaten a lot of carbs prior to embarking on this diet this might mean that it will take you a few days to adjust to your new style of eating and for your body to send the right signals that you are feeling full. Don't worry about this, after a few days chances are that you will eat less than you used to as you feel fuller sooner.

Fat will be the nutrient that you can eat as much as want of, however, this means only eating until you feel full and satisfied. If you go past the point of feeling full you will still overeat and undo all the goodness that you have done for your body. Listening to your body and knowing when to stop is the key!

One important thing to bear into consideration is to become more mindful when you are eating and to eat slowly, rather than devouring your food within 10 minutes because you feel that there is no time to eat. Eating in a rush and quite frankly 'shoving it in' means that by the time your brain has had the chance to signal your stomach that you have had enough food to satisfy all your physiological needs, thank you very much, you will have most likely overdone it already. You will be surprised to find that when you take it down a notch with the speed eating that you might want to eat far less than anticipated because before you know it you will feel full.

If your day really is that manic and lunch is a 10-minute affair or even takes place behind the wheel of your car (we live in busy, demanding times, we know, and sometimes your boss, children, the situation might not appreciate the eat slowly mantra) the good thing about a high-fat diet is that you can rest assured that once you feel full you will do so for longer. So if your lunch break is hectic eat slightly less at first. The feeling of fullness will come and if this is something that worries you, ensure that you have a snack handy that might fill that hole after all if you get the afternoon slump or your tummy starts rumbling again.

Do not eat more than 2 – 3 portions of protein from the non-dairy list per day. Remember, this is a high-fat diet, not a high-protein diet.

As you should not consume more than 50 grams of carbohydrates per day make sure that you do not eat any items on the 'no-no foods' list. Eating the recommended items from the vegetables and fruit list will help you get there, as will incorporating dairy products, nuts and seeds. This is not a no-carb but a low-carb diet so some carbs are necessary for you to consume and as we do not tend to eat every part of the animal in the UK in the same way as other cultures might do, which addresses their requirements for vitamins and minerals - think seal's eyeball as good sources of these (yum!) - it is important that you incorporate veggies and some fruit in your diet. We will give you a daily multi-vitamin and mineral supplement to

avoid any potential shortfalls, but this can only take you so far. The majority of your nutrients should come from your diet!

## Suitable snacks

Overall, after a period of adjustment, we would expect for you not having to snack so much, if at all, as a low-carb, high-fat diet should keep you feel fuller for longer. However, do not despair there are some good snack options out there for you. It just won't be that piece of cake or a packet of crisps.

- Avocados – full of high-quality fats, creamy and delicious
- Canned mackerel in tomato sauce – just be careful with your breath with this one. And read the label to see if any sugars are lurking in that tomato sauce!
- Eggs (hardboiled) – ultimate, easy snack. Put a bit of salt on if you want!
- Slices of ham or salami and cheese rolled up and maybe some cheese spread on – think charcuterie in your favourite tapas restaurant. However, do remember to not eat more than 70 grams of these types of processed meats per day!
- Home-made low-carb crackers – hardly any carbs but lots of fibre. We will give you the recipe
- Nuts – again, pure natural goodness. But if you want some variation, we have some great and easy recipes on how you can flavour these.
- Olives – easy to get hold of with the added benefit of containing high-quality fats
- A piece of cheese – great source of calcium
- Pork scratchings – these can be a good alternative to that packet of crisps
- Some vegetables and a suitable dip – cucumber, celery, peppers, carrots. Again we have some great recipes for dips.

## Some staples and alternatives to favourites on a very low-carb diet

- Almond flower – just because you are going low-carb does not mean that you cannot do some baking or have pancakes
- Bone broth – full of magnesium and potassium and other minerals to do your body good
- Bread – we will give you recipes for keto-bread if you can't be without.
- Coconut flower – Another low-carb alternative in cooking and baking
- Psyllium – To make keto crackers, porridge and increase fibre intake
- Pasta – Have spiralised vegetables, such as courgette instead. You can either make these yourself by investing in a spiralizer or they are now readily available from a number of supermarkets and discounters. They have all caught on to this trend.
- Porridge – A breakfast favourite of yours? Although oats are not allowed on low-carb there are some tasty alternatives, and we have the recipes to give you.
- Potatoes – Love mash? How about some caulimash instead? Or other vegetable mashes.
- Rice – A must when you are having curry? Or love risotto? Have cauliflower rice instead. You've guessed it – we do have some recipes for you.

## Hydration

This is an important one that often gets overlooked. You should drink 6-8 glasses (200ml or medium-sized) per day to ensure staying hydrated. This will also help your body to cope with a potential increase in dietary fibre following a healthy diet (see above). However, you should stick to water, unsweetened black or herbal teas and black coffee or coffee with small amounts of full fat milk or cream. (We also have a recipe for *bullet proof* coffee.) Be careful with flavoured milks as these also tend to contain a lot of sugar which will catapult you right out of the max. 50g of carbs per day zone! If you think that plain water is too boring there are some ways (and recipes) to make this more interesting, such as adding slices of citrus fruit and/or mint or cucumber for example. If you are struggling with the taste of tea and coffee without sugar there are some alternatives on the market that you can use sparingly. These can be found in the section about sugar on page 21.

You should also not drink any fruit juices as the natural sugar contained in fruits has been freed through the process of liquidisation. This means it is more easily digested and can raise your blood sugar a lot quicker. The fruit sugar contained in fruit (fructose) can actually not be metabolised by our bodies and if not turned into energy will be converted into body fat. In addition, this free sugar can contribute to dental decay.

## Alcohol

Small glasses of dry wine are permitted and these contain about 2 grams of carb per glass. So enjoy your glass of wine, but do not overdo it! Spirits such as whisky, brandy, vodka, sugar-free cocktails are also permitted. But be careful not to mix these with soda as this will make your drink too sugary. Beer (think liquid bread!), cider and mixer drinks (even that G&T) with sugary sodas or alcopops are not allowed as these contain far too many carbohydrates, which will be broken down fairly quickly and end up in your blood stream and cells. Bye-bye low-carb diet! Check the labels of diet versions of diet sodas to see whether they contain 0 sugars/carbs and these might be an alternative. However, do bear in mind what we said about artificial sweeteners and their potential to still induce sugar cravings. Furthermore, there is currently debate on whether some artificial sweeteners still elevate your insulin levels. We therefore strongly advise you to proceed with caution here at least whilst you are part of our cohort.

## Learning to read labels and nutritional information

This is an important one when food shopping and eating out in places that provide you with information on the nutritional content of their dishes, for example fast food restaurants and pubs. If you are unsure check out the label for the carbohydrate content of the food. Avoid anything with more than 5gram per 100 grams of carbs or more than 5 % of carbs.

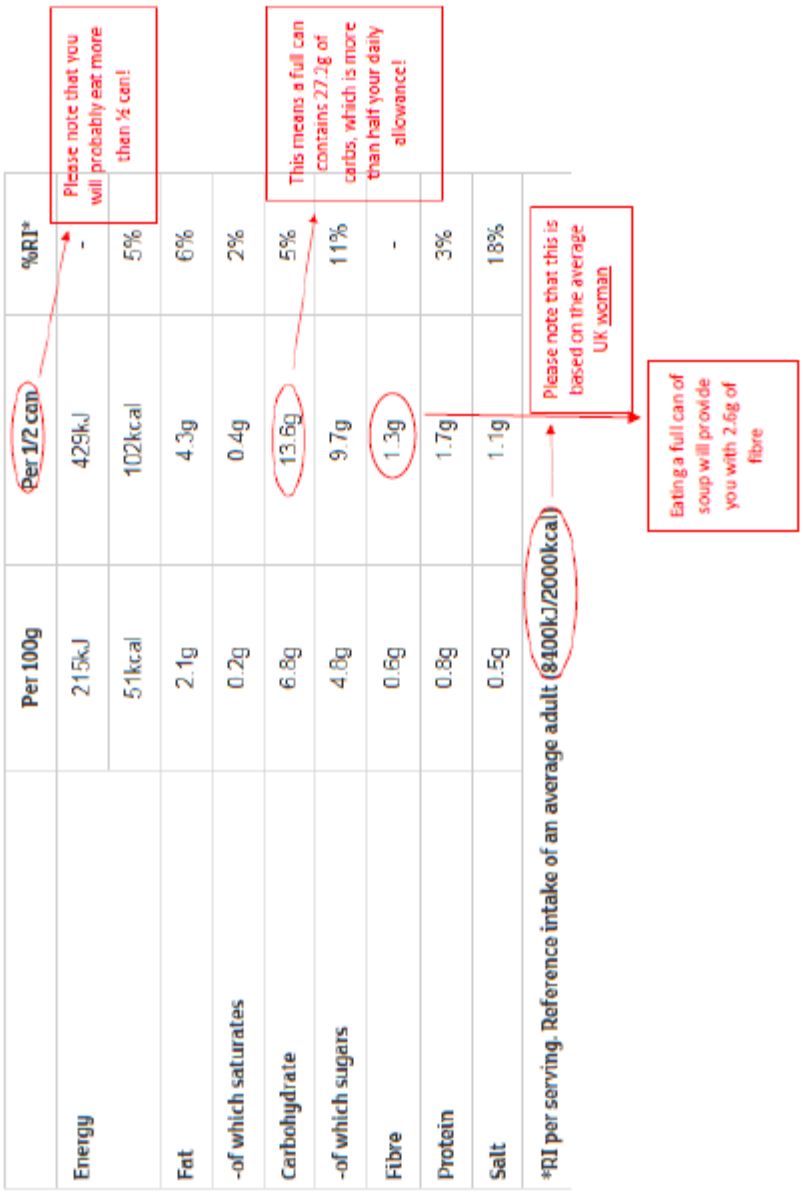
### Label reading

In the UK food labels can be found at the back and depending on the manufacturer or retailer also at the front of the packaging.

Labels at the back of the packaging

**Below is the back of pack nutrition label for Heinz Tomato soup as an example.**

**Ea**




| | Per 100g | Per 1/2 can | %RI* |
|---|---|---|---|
| Energy | 215kJ | 425kJ | - |
| | 51kcal | 102kcal | 5% |
| Fat | 2.1g | 4.3g | 6% |
| -of which saturates | 0.2g | 0.4g | 2% |
| Carbohydrate | 6.8g | 13.6g | 5% |
| -of which sugars | 4.8g | 9.7g | 11% |
| Fibre | 0.6g | 1.3g | - |
| Protein | 0.8g | 1.7g | 3% |
| Salt | 0.5g | 1.1g | 18% |

*RI per serving. Reference intake of an average adult (8400kJ/2000kcal)

There are a few things to be aware of:

The manufacturer's portion size guide might actually not be a realistic reflection of what you might actually eat yourself and it can be very easy to underestimate the amount of carbohydrates you are consuming.

Another thing to take into consideration is the quality of carbohydrates you are eating. The label will not tell what source the total sugars contained in foods derive from and can be a combination of natural sugars found in fruits (fructose) and milk (lactose) where applicable and added sugars.

This is where looking at the ingredients list (also a the back of pack) might necessary as sugar comes under many different names and there might be some surprises in store for you.

As a general rule, the closer an added sugar is towards the top of the ingredients list the more of it will be contained in the product. There might also be different types of added sugar in the same product.

All of these that you might spot in the ingredients list are actually added sugars

> agave sugar, brown sugar, cane sugar, dextrose, fructose, fruit juice concentrate, glucose, golden syrup, HFCS/high fructose corn syrup, honey, hydrolysed starch, invert sugar, isoglucose, levulose, maltose, modified starch molasses, sucrose, syrups (sucrose, glucose, malt, corn, maple), treacle,

Once a product crosses specific thresholds for specific nutrients it will either be classed as 'medium' or 'high' in sugar or other nutrients (see below).

| | Low | Medium | High |
|---|---|---|---|
| **Total sugars** | 5g per 100g product (or 5%) | More than 5g but less than 22.5 per 100g product (between 5% and 22.5%) | More than 22.5g per 100g product (or 22.5%) |
| **Salt** | 0.3g of salt or less per 100g (or 0.1g sodium) | More than 0.3g of salt (0.1g sodium) but less than 1.5g of salt (0.6g sodium) per 100g product | More than 1.5g of salt per 100g (or 0.6g sodium) |

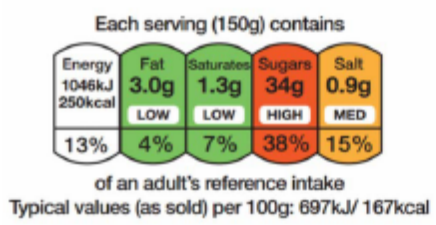
If the portion size means that more than 25g of sugar would be consumed with one portion the product is automatically classed as 'high sugar'.

For drinks containing total sugar the rule is that 2.5g per 100ml is classed as 'low', between 2.5 and 11.25g per 100ml is classed as 'medium' and above 11.25g per 100ml is 'high'. If the

portion size of a drink means that more 13.5g of sugar would be consumed the product is automatically classed as 'high sugar'.

### Front of pack labels

Some retailers and manufacturers display a food label at the front of the pack highlighting whether some of the crucial nutrients are contained in the foods to low, medium or high levels. This is called the 'traffic light system'. The main nutrient that you will need to look out for is the 'sugars' category. You do not need to concern yourself with the 'fat' and 'saturates' categories on a low-carb diet as the permitted amounts are different from the standards applied to the traffic light labels. These are based on the UK dietary guidelines.




### Interpreting nutritional information provided by fast food restaurant and pub chains

A number of high street fast food outlets and pub chains publish nutritional information on typical serving sizes of their dishes that you can access online, download or on request at the restaurant.

Being aware of the nutritional content of some of these dishes is helpful if you tend to eat out more often as it can be difficult in this case to stick to a low-carb diet and you might unknowingly jeopardise all your efforts.

It is also great to make you aware of how much individual components will add up. Watch out for hidden carbs, including starches and sugars in sauces, salad dressings and coatings.

Some example nutrition guides are attached to this guide to give you an idea what to look out for. However, these are by no means meant as endorsements of particular eateries.

## Tips for Eating Out on a low-carb, high-fat diet

Eating out whilst on a low-carb diet can be quite straight forward depending on the type of cuisine that you choose and very often it is easy to make your meal low-carb friendly. We have also provided you with example nutritional information from some of the pub and restaurant chains in the UK to give you examples what to look out for. In general, all chains should have this information readily available for interested diners in the restaurants and on their websites. If you would like us to try and find the nutritional information for a particular place, please do let us know.

- Many restaurants and pubs now will allow you to swap your potatoes and chips with a side salad.
- If you fancy a burger just have it without the bun or if you feel comfortable doing so ask for it to be wrapped in a large lettuce leaf instead (so you can still pick it up)

- Instead of breaded/battered fish or chicken have the steamed or roasted versions instead.
- If you get a Mexican or a Subway takeaway ask for the salad version of the dish rather than the wrap/burrito.
- Be careful with sauces and gravies as these might contain flour (and carbs!). In order to control how much (and if) you want to eat any of this ask for it to be served on the side rather than on your plate.
- If you fear that you might feel a bit hungry still after you have eliminated the starchy foods from your restaurant plate, ask for (extra) butter or olive oil to make up for this. Some people following a low-carb take a small bottle of olive oil with them when eating out just in case.
- If crave a third course see if there is a cheese platter on the menu (without the crackers!) instead of opting for pudding.
- It might be difficult to eat in Indian or Chinese buffet restaurants or takeaways whilst you are participating in the CALIBER study. Other research undertaken by our nutrition team has shown these dishes to be very high in added sugars. You definitely need to avoid the sweet and sour chicken! However, Indian creamy curries and kebabs might be a good option.
- Go easy on the condiments as ketchup, cocktail and BBQ sauces can contain a lot of added sugar.
- If you know that you have been invited to a dinner party and you don't want to offend, be careful of you carb intake earlier during the day. This way you can at least try and contain some of the damage.
- Otherwise, if your host is understanding give them a fair warning, which should be much appreciated. Some low-carbers excuse their avoidance of starchy foods with stomach issues. If you think that you might not be able to eat enough at the dinner party, have a snack at home before you leave.
- Pizza is a harder one! It should be avoided during your eight weeks on our study. If you really crave pizza, you can use your own using an alternative base. Please see the recipe that we have provided for this.

## Appendix - Helpful App to help you to stick to a low-carb diet

### Change4Life – Sugar smart App

Change4Life is a public health initiative run by Public Health England. This app lets you scan the barcodes of about 87,000 food products available from UK major manufacturers and retailers. It focuses on the amount of free sugar in these foods and uses a traffic light system to let you know whether these would be high in the nutrients. A good way to find out about any unexpected sugars in products that you might want to buy.


The app can be downloaded for free from the Appstore and GooglePlay.

# Appendix - Examples nutritional information fast food, pub and restaurant chains

## ASK

Italian

| Recipe / Dish | Menu selections | Per 100g** Energy KJ | Kcal | Fat (g) | Saturates (g) | Carbs (g) | sugars (g) | Protein (g) | Salt (g) | Per Serving** Energy KJ | Kcal | Fat (g) | Saturates (g) | Carbs (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Italian Olives | Breads / Nibbles | 673 | 163 | 15 | 2.0 | 3.8 | 0.5 | 1.0 | 3.0 | 841 | 204 | 19 | 2.5 | 4.8 |
| Rosemary & Sea Salt Bread | Breads / Nibbles | 1049 | 251 | 5.7 | 0.8 | 41 | 2.8 | 7 | 1.1 | 2089 | 499 | 11 | 1.5 | 81 |
| Garlic Bread Speciale | Breads / Nibbles | 1134 | 270 | 11 | 6.4 | 33 | 2.4 | 11 | 1.1 | 3069 | 729 | 29 | 17 | 89 |
| Garlic Bread | Breads / Nibbles | 1144 | 274 | 7.8 | 1.9 | 38 | 2.6 | 6.6 | 1.1 | 2455 | 588 | 17 | 4.1 | 82 |
| Garlic Bread with Mozzarella | Breads / Nibbles | 1153 | 276 | 11 | 4.6 | 29 | 2.2 | 11 | 1.2 | 3281 | 784 | 31 | 13 | 83 |
| Cheese Fonduta | Breads / Nibbles | 1121 | 264 | 11 | 4.2 | 33 | 1.2 | 9.7 | 1.2 | 3269 | 771 | 33 | 12 | 96 |
| Antipasti - The Warm One | Starters & Shares | 934 | 223 | 13 | 3.3 | 17 | 1.5 | 8.8 | 0.8 | 5946 | 1422 | 83 | 21 | 109 |
| Antipasti - The Cheese One | Starters & Shares | 1143 | 274 | 17 | 7.9 | 22 | 4.7 | 9.9 | 0.8 | 5787 | 1388 | 85 | 40 | 111 |
| Antipasti - The Meat One | Starters & Shares | 1123 | 268 | 16 | 3.4 | 22 | 1.0 | 10 | 1.7 | 4776 | 1140 | 70 | 15 | 92 |
| Antipasti - The Mixed One | Starters & Shares | 1181 | 284 | 16 | 7.2 | 21 | 4.5 | 9.5 | 1.0 | 6268 | 1508 | 98 | 38 | 111 |
| Mussel Marinara | Starters & Shares | 448 | 106 | 6.4 | 0.6 | 7.9 | 1.6 | 3.9 | 0.6 | 1564 | 371 | 22 | 2.1 | 28 |
| Bruschetta | Starters & Shares | 692 | 165 | 8.5 | 1.6 | 18 | 2.0 | 4.5 | 1.0 | 1343 | 321 | 17 | 3.2 | 34 |
| Calamari (1 Serving) | Starters & Shares | 842 | 201 | 14 | 1.5 | 11 | 0.9 | 7.3 | 1.0 | 1507 | 360 | 25 | 2.8 | 19 |
| Mushrooms Al Forno | Starters & Shares | 1391 | 330 | 27 | 5.3 | 18 | 1.3 | 6.3 | 0.6 | 2274 | 540 | 43 | 8.6 | 29 |
| Meatballs | Starters & Shares | 815 | 195 | 9.7 | 3.5 | 16 | 2.1 | 12 | 1.4 | 1223 | 292 | 15 | 5.3 | 24 |
| Insalata Caprese | Starters & Shares | 776 | 187 | 15 | 7.0 | 1.6 | 1.6 | 11 | 0.5 | 1622 | 391 | 32 | 15 | 3.4 |
| Croquettes | Starters & Shares | 888 | 213 | 14 | 2.9 | 14 | 1.4 | 6.8 | 0.9 | 1760 | 423 | 28 | 5.7 | 29 |
| Butterfly King Prawns | Starters & Shares | 923 | 221 | 11 | 3.4 | 14 | 1.7 | 9.3 | 1.0 | 1737 | 416 | 21 | 6.3 | 27 |
| Melanzane al Forno | Starters & Shares | 631 | 151 | 12 | 3.6 | 5 | 2.5 | 5.8 | 0.6 | 1259 | 301 | 24 | 7.3 | 10 |
| Tagliatelle Carbonara | Fresh Pasta | 497 | 232 | 15 | 8.1 | 15 | 0.9 | 9.8 | 0.9 | 4535 | 1079 | 69 | 38 | 70 |
| Aragosta e Gamberoni | Fresh Pasta | 628 | 149 | 4.9 | 1.8 | 17 | 2.0 | 8.5 | 1.0 | 2756 | 652 | 21 | 8.1 | 75 |
| Beef Tortellini | Fresh Pasta | 513 | 123 | 5.5 | 2.8 | 13 | 2.0 | 6.4 | 0.9 | 1619 | 387 | 18 | 8.9 | 39 |
| Funghi Misti | Fresh Pasta | 596 | 142 | 7.2 | 4.1 | 16 | 3.4 | 4.7 | 0.4 | 2586 | 616 | 33 | 18 | 76 |
| Rigatoni con Zucchine e Pesto - without chicken | Pasta - Classic | 1132 | 268 | 16 | 3.4 | 27 | 2.8 | 7.1 | 1.0 | 3376 | 895 | 52 | 12 | 91 |
| Fettuccine Bolognese | Pasta - Classic | 804 | 190 | 5.9 | 1.3 | 25 | 3.1 | 8.6 | 0.9 | 2929 | 692 | 21 | 4.8 | 87 |
| Spaghetti al Pomodoro | Pasta - Classic | 583 | 139 | 5.0 | 1.6 | 20 | 2.7 | 4.3 | 0.5 | 2819 | 672 | 24 | 7.8 | 98 |
| Linguine Carbonara | Pasta - Classic | 1209 | 287 | 16 | 9.0 | 28 | 3.0 | 9.5 | 0.7 | 4354 | 1035 | 58 | 32 | 103 |
| Linguine con Frutti di Mare | Pasta - Classic | 584 | 137 | 4.3 | 0.5 | 18 | 1.6 | 7.2 | 1.5 | 2977 | 703 | 22 | 2.4 | 89 |
| Ravioli Marittimi | Pasta - Classic | 721 | 173 | 7.9 | 3.0 | 18 | 5.6 | 7.5 | 0.8 | 2546 | 611 | 28 | 11 | 63 |
| Rigatoni di Manzo Piccante | Al Forno | 720 | 171 | 8.1 | 3.0 | 15 | 2.3 | 9.2 | 0.8 | 3979 | 945 | 45 | 17 | 85 |
| Penne al Pollo della Casa | Al Forno | 634 | 151 | 7.3 | 3.7 | 14 | 1.8 | 7.7 | 0.4 | 3525 | 837 | 41 | 21 | 76 |
| Lasagne | Al Forno | 692 | 166 | 10 | 4.0 | 11 | 2.8 | 6.4 | 0.4 | 3096 | 742 | 47 | 18 | 51 |
| Melanzane al Forno - Without Side Salad | Al Forno | 631 | 151 | 12 | 3.6 | 5 | 2.5 | 5.8 | 0.6 | 2547 | 609 | 49 | 14 | 20 |
| Risotto Pescatore | Risotto | 560 | 133 | 3.3 | 1.1 | 19 | 1.3 | 5.9 | 0.9 | 3408 | 812 | 20 | 6.6 | 118 |
| Risotto con Pollo e Funghi | Risotto | 579 | 138 | 4.0 | 2.0 | 19 | 1.2 | 5.7 | 1.1 | 3420 | 818 | 24 | 12 | 114 |
| Risotto Rosso | Risotto | 580 | 139 | 5.0 | 2.5 | 20 | 1.7 | 0.5 | 1.0 | 3333 | 797 | 29 | 14 | 116 |
| Pork Belly Porchetta | Meat & Fish | 813 | 196 | 15 | 5.5 | 5.8 | 2.8 | 10 | 0.4 | 4673 | 1143 | 87 | 32 | 35 |
| Pollo Prosciutto | Meat & Fish | 474 | 112 | 4.5 | 1.9 | 7.0 | 1.0 | 12 | 0.8 | 2370 | 562 | 22 | 9.5 | 35 |
| Sea Bass al Forno | Meat & Fish | 431 | 103 | 5.3 | 2.4 | 5.7 | 0.8 | 8.4 | 0.8 | 2335 | 560 | 29 | 13 | 31 |
| Prima Pizza Salami Misti | Pizza & Calzone | 856 | 203 | 8.9 | 4 | 21 | 2.3 | 11 | 1.2 | 4255 | 1011 | 44 | 20 | 103 |

## Brewers Fayre

| Product/ Dish Description | Nutrition Information Per Portion | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | kJ | kcal | Fat (g) | Saturates (g) | Carbohydrate (g) | Sugars (g) | Protein (g) | Salt (g) |
| **DAYTIME VALUE & SNACKS MENU** | | | | | | | | |
| **STARTERS** | | | | | | | | |
| TOMATO SOUP | 980 | 234 | 7.7 | 3.3 | 33.0 | 11.8 | 5.7 | 1.8 |
| CRISPY POTATO DIPPERS | 2045 | 488 | 26.6 | 12.1 | 34.0 | 2.7 | 23.6 | 1.7 |
| BUBBLE & SQUEAK | 1458 | 348 | 24.4 | 10.9 | 19.5 | 3.4 | 10.4 | 1.4 |
| GARLIC & HERB BREADED MUSHROOMS | 1627 | 389 | 17.3 | 1.9 | 53.0 | 6.7 | 8.7 | 1.5 |
| **MAINS** | | | | | | | | |
| SMOTHERED CHICKEN | 3991 | 953 | 45.3 | 15.1 | 79.0 | 11.9 | 55.4 | 4.5 |
| LASAGNE | 2653 | 634 | 28.1 | 12.1 | 64.3 | 17.0 | 27.9 | 3.1 |
| GRILLED GAMMON STEAK WITH EGGS | 3841 | 917 | 37.8 | 12.5 | 62.5 | 5.6 | 80.1 | 4.5 |
| GRILLED GAMMON STEAK WITH PINEAPPLE | 3655 | 873 | 31.3 | 10.8 | 81.2 | 26.4 | 67.8 | 4.2 |
| GRILLED GAMMON STEAK WITH ONE OF EACH | 3748 | 895 | 34.6 | 11.6 | 71.9 | 16.0 | 74.0 | 4.3 |
| FISH & CHIPS WITH PEAS | 5227 | 1248 | 73.2 | 12.3 | 107.4 | 6.9 | 37.5 | 1.7 |
| FISH & CHIPS WITH MUSHY PEAS | 5386 | 1286 | 73.3 | 12.4 | 113.9 | 5.6 | 39.6 | 2.6 |
| BATTERED GIANT HADDOCK & CHIPS WITH PEAS | 4501 | 1075 | 59.3 | 16.2 | 86.3 | 7.7 | 47.4 | 1.7 |
| BATTERED GIANT HADDOCK & CHIPS WITH MUSHY PEAS | 4660 | 1113 | 59.4 | 16.3 | 92.8 | 6.4 | 49.7 | 2.6 |
| MEXICAN BEEF CHILLI | 2999 | 716 | 18.6 | 5.4 | 97.1 | 5.7 | 28.7 | 2.2 |
| THREE CHEESE CRUSTLESS QUICHE | 2390 | 571 | 36.4 | 18.0 | 42.2 | 10.6 | 19.7 | 1.2 |
| THE SOUTH WESTERN BURGER | 4105 | 980 | 47.6 | 11.0 | 106.5 | 12.4 | 29.6 | 2.4 |
| BEEF, CHEESE & MUSHROOM BURGER | 4846 | 1157 | 64.5 | 23.0 | 93.3 | 12.3 | 48.7 | 2.5 |
| GRILLED CHICKEN & BACON SALAD | 1816 | 434 | 19.0 | 5.6 | 12.4 | 11.3 | 50.9 | 4.7 |
| CHICKEN TIKKA CURRY | 3614 | 863 | 24.0 | 6.2 | 113.1 | 25.6 | 45.3 | 3.7 |
| BREADED WHOLETAIL SCAMPI WITH PEAS | 3885 | 928 | 44.7 | 8.3 | 107.0 | 7.0 | 24.2 | 3.9 |
| BREADED WHOLETAIL SCAMPI WITH MUSHY PEAS | 4044 | 966 | 44.8 | 8.4 | 113.6 | 5.7 | 26.5 | 4.8 |
| SMOKY PAPRIKA CHICKEN | 1802 | 430 | 14.7 | 4.1 | 31.9 | 19.1 | 41.4 | 2.2 |
| SAUSAGE, EGG & CHIPS | 4170 | 996 | 57.1 | 18.9 | 74.2 | 7.4 | 44.0 | 2.5 |
| SWEET POTATO & FETA LASAGNE | 3106 | 742 | 39.1 | 15.8 | 69.9 | 17.8 | 24.2 | 3.0 |
| HOT 'N' SPICY VEGGIE NACHO BURGER | 5160 | 1233 | 61.8 | 19.9 | 142.3 | 17.9 | 23.0 | 3.0 |
| HAM & CHEESE SANDWICH WITH WHITE BREAD | 2613 | 624 | 28.3 | 12.6 | 55.8 | 3.0 | 35.2 | 3.5 |

Typical values per portion

| | Energy, kJ | Energy, kcal | Total fat, g | Of which Saturated, g | Carbohydrates, g | of which Sugars, g | Fibre, g | Protein, g | Salt, g | Average portion size (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| **MEAT** | | | | | | | | | | |
| Bacon | 267.2 | 64.5 | 5.40 | 2.2 | 0.2 | 0.0 | 0.1 | 3.9 | 0.97 | 11.3 |
| Beef Burger Patty | 745.4 | 179.0 | 13 | 5.1 | 0.1 | 0.1 | 0.3 | 16 | 0.12 | 81.5 |
| Hot Dog | 974.2 | 235.0 | 20 | 8.5 | 0.5 | 0.7 | 0.4 | 13 | 1.49 | 88 |
| **BUN** | | | | | | | | | | |
| Burger bun | 959.4 | 227.7 | 7.20 | 2.46 | 36 | 5.93 | 1.34 | 5.76 | 0.47 | 73.8 |
| Hot dog bun | 1067.8 | 253.2 | 7.90 | 2.66 | 40 | 6.99 | 1.42 | 6.26 | 0.55 | 82.3 |
| **FRIES** | | | | | | | | | | |
| Little FRIES - COOKED IN PEANUT OIL | 2980.2 | 716.0 | 44 | 5.81 | 72 | 0.79 | 7.66 | 11 | 1.14 | 264.2 |
| Regular FRIES - COOKED IN PEANUT OIL | 4823.3 | 1158.8 | 72 | 9.41 | 116 | 1.28 | 12 | 18 | 1.85 | 427.6 |
| Large FRIES - COOKED IN PEANUT OIL | 7178.6 | 1724.6 | 107 | 14 | 173 | 1.91 | 18 | 26 | 2.75 | 638.4 |
| Cajun Seasoning | 51.0 | 12.2 | 0.20 | 0.03 | 1.77 | 0.65 | 0.65 | 0.49 | 0.70 | 4.25 |
| **TOPPINGS** | | | | | | | | | | |
| BBQ Sauce | 86.3 | 20.4 | 0.08 | 0.02 | 4.11 | 3.95 | 0.39 | 0.23 | 0.35 | 15 |
| Cheese (1Slice) | 300.9 | 71.9 | 5.81 | 3.72 | 0.58 | 0.58 | 0.00 | 3.78 | 0.72 | 19.4 |
| Green Peppers | 8.3 | 1.9 | 0.02 | 0.01 | 0.43 | 0.22 | 0.16 | 0.08 | 0.00 | 9.25 |
| Grilled Mushrooms | 51.2 | 12.1 | 0.13 | 0.03 | 1.97 | 0.03 | 0.75 | 1.14 | 0.01 | 32.8 |
| Hot Sauce | 8.3 | 1.9 | 0.05 | 0.01 | 0.14 | 0.02 | 0.12 | 0.19 | 0.65 | 7.75 |
| HP Brown Sauce | 63.3 | 12.5 | 0.01 | 0.01 | 3.47 | 2.83 | n/a | 0.11 | 0.16 | 12.3 |
| Jalapeno Peppers | 4.4 | 1.0 | 0.03 | 0.01 | 0.56 | 0.33 | 0.22 | 0.07 | 0.02 | 8 |
| Tomato Ketchup | 72.9 | 17.1 | 0.02 | 0.00 | 3.89 | 3.82 | n/a | 0.20 | 0.30 | 18.8 |
| Lettuce | 8.6 | 2.9 | 0.02 | 0.00 | 0.27 | 0.00 | 0.18 | 0.14 | 0.00 | 15 |
| Mayonnaise | 441.2 | 107.3 | 12 | 0.86 | 0.49 | 0.49 | n/a | 0.13 | 0.24 | 16.3 |
| Mustard | 19.4 | 4.7 | 0.24 | 0.05 | 0.27 | 0.05 | 0.17 | 0.27 | 0.17 | 8.3 |
| Onions | 31.9 | 7.0 | 0.02 | 0.00 | 1.58 | 0.74 | 0.30 | 0.19 | 0.00 | 17.5 |
| Grilled Onions | 45.2 | 10.6 | 0.11 | 0.03 | 2.47 | 1.46 | 0.80 | 0.37 | 0.00 | 20.6 |
| Pickles | 9.0 | 1.9 | 0.00 | 0.00 | 0.27 | 0.24 | 0.36 | 0.21 | 0.57 | 23.8 |
| Relish | 104.5 | 24.6 | 0.06 | 0.03 | 5.62 | 4.64 | 0.19 | 0.06 | 0.22 | 16 |
| Tomatoes | 24.6 | 5.6 | 0.04 | 0.00 | 1.21 | 1.21 | 0.40 | 0.04 | 0.00 | 40.3 |

Watch out when you are adding extras to your burger

## Harvester

| STARTERS AND SHARERS | Energy (KJ) | Energy (Kcal) | Fat (g) | Saturated Fat (g) | Carbohydrate (g) | Sugars (g) | Protein (g) | Salt (g) |
|---|---|---|---|---|---|---|---|---|
| Potato Skins with Bacon & Cheese | 1,934 | 460 | 26.2 | 13.5 | 32.2 | 2.1 | 22.8 | 2.15 |
| BBQ Chicken Wings | 1,593 | 379 | 18.4 | 4.8 | 20.3 | 18.6 | 32.8 | 1.98 |
| Crispy Coated Chicken Bites | 1,683 | 401 | 17.9 | 3.2 | 36.9 | 18.3 | 22.9 | 2.21 |
| Cheese Popits with Salsa V | 1,759 | 419 | 25.9 | 10.1 | 33.1 | 4.4 | 12.2 | 1.46 |
| Spicy Crackerjack King Prawns | 1,359 | 324 | 13.3 | 3.1 | 40.0 | 13.6 | 9.3 | 2.02 |
| Breaded Mushrooms V | 2,363 | 563 | 39.2 | 6.0 | 43.6 | 2.2 | 8.1 | 1.88 |
| Chicken & Chorizo skewers NEW | 1,990 | 474 | 36.5 | 12.4 | 6.7 | 5.5 | 28.3 | 1.81 |
| Ultimate Nachos V | 3,633 | 865 | 48.7 | 15.8 | 82.0 | 4.8 | 20.3 | 3.03 |
| *Why not add Three Bean Chilli V* | 464 | 110 | 3.0 | 0.4 | 15.1 | 6.8 | 4.2 | 0.78 |
| *Or add BBQ Pulled Pork* | 1,298 | 309 | 10.1 | 3.3 | 40.4 | 38.7 | 13.6 | 1.43 |
| Creamy Tomato & Basil Soup V NEW | 1,268 | 302 | 5.7 | 2.7 | 53.5 | 4.9 | 8.0 | 2.26 |
| Sticky Duck Wings | 1,839 | 438 | 19.1 | 5.1 | 35.5 | 32.5 | 29.2 | 2.69 |
| Fish Basket NEW | 2,994 | 713 | 45.2 | 7.5 | 54.5 | 7.5 | 21.7 | 4.00 |
| Cheesy Garlic Bread Board V NEW | 5,795 | 1,380 | 92.6 | 31.8 | 108.4 | 15.3 | 34.0 | 5.15 |
| **CHICKEN** | **Energy (KJ)** | **Energy (Kcal)** | **Fat (g)** | **Saturated Fat (g)** | **Carbohydrate (g)** | **Sugars (g)** | **Protein (g)** | **Salt (g)** |
| Harvester's Famous 1/2 Rotisserie Chicken | 1,885 | 449 | 22.9 | 5.0 | 2.4 | 3.5 | 57.1 | 1.58 |
| Whole Rotisserie Chicken NEW | 3,525 | 839 | 41.2 | 10.0 | 2.4 | 4.0 | 113.5 | 3.07 |
| Chargrilled Chicken Breast | 1,126 | 268 | 9.1 | 1.0 | 2.5 | 3.1 | 43.0 | 1.48 |
| Triple Chicken | 2,763 | 658 | 27.7 | 5.7 | 13.6 | 3.6 | 87.3 | 2.94 |
| Chicken Skewer | 1,879 | 447 | 25.9 | 6.0 | 2.5 | 3.3 | 49.7 | 0.92 |
| Salsa Chicken & Pepper Stack | 1,588 | 378 | 14.0 | 1.2 | 11.4 | 11.4 | 49.0 | 1.90 |
| HARVESTER RECOMMENDS BBQ Brushed & Basted | 4,487 | 1,068 | 43.8 | 9.3 | 103.9 | 47.4 | 63.0 | 3.67 |
| HARVESTER RECOMMENDS Piri Piri Brushed & Basted | 3,952 | 941 | 48.0 | 9.6 | 63.8 | 8.0 | 62.1 | 6.22 |
| Garlic & Parsley Brushed & Basted | 6,259 | 1,490 | 40.3 | 12.9 | 59.9 | 73.1 | 69.8 | 3.1 |
| Farmer Rikki's Hot Chilli Sauce Brushed & Basted | 4,117 | 980 | 52.0 | 9.9 | 62.7 | 5.8 | 63.1 | 2.99 |

# Dietary Guidelines Group (High-carb)



CALIBER study Merseyside

Liverpool John Moores University

School of Sport Studies, Leisure and Nutrition

Tanja Harrison BSc (Hons) ANutr AFHEA

SEDA

## Contents

## Quick guide

For the next eight weeks you have been allocated to the high-carb group of the CALIBER study, which means that you will be asked to follow the UK dietary recommendations.

We will undertake a variety of assessments with you when you come to see us in our laboratory at IM Marsh campus on three occasions, including blood samples, body composition, dietary intake and impact on this diet on food cravings and cognition. We will also ask you to wear a wrist-worn physical activity monitor on three occasions.

These guidance notes will give you further information on which types of food to eat, what makes a portion size for carbohydrates, fats, proteins and fruits and vegetables and how to read food labels and become aware of the nutritional content of out-of-home cooked meals to support your efforts to stick to this eating plan.

In this booklet, you will also find the names and contact details of the people involved in this research.

## Introduction

Hello and welcome to the CALIBER study! Nice to have you on board.

This booklet has been designed to be your companion over the next eight weeks whilst you are part of our cohort and to support you to consume a healthy diet whether you are cooking at home, buying ready-made meals or eating out. There are certain rules that can be applied to all of these situations.

We will also give you information on the purpose of our study, what to expect during your visits to our laboratories and once the study has finished and we have analysed the results. This means that not only will you help an important research cause but you can also find out how you and your body did over the course of these eight weeks and how your body composition, blood profile, food cravings and cognition might have been affected.

We hope that you will find your time on the study interesting, inspiring, motivating and delicious.

## What do we want to find out?

Maybe you have followed the news over these past couple of years and noticed that there is a lot of controversy and discussion about what makes a diet healthy. The debate has been particularly heated around the issue of carbohydrates. Whilst many public health officials have argued that the vast majority of the population does not follow the dietary UK guidelines (which can be classed as high-carbohydrate, moderate-fat) and that this is the root cause of the UK's problem with ill-health, obesity and diseases such as heart disease and type 2 diabetes, others claim that these guidelines have caused these problems to begin with. The latter group advocates to reduce the amounts of carbohydrates we consume as a nation and for the guidelines to be re-written.

A third group suggests that it is far more complicated than this and that how we react to carbohydrates is actually far more personal and not one-size-fits-all, but some people might be better off on low-carb diets whilst others fare better on high-carb diets in terms of cardiometabolic health.

This is where our study comes in, CALIBER – Carbohydrates, lipids and biomarkers of traditional and emerging cardiometabolic risk factors. We want to compare the effects of consuming either a high-carb or a low-carb diet on these risk factors. The reason why we have asked you to join us is because you showed some slightly elevated risk markers for these illnesses, albeit still at a stage where these should be easy to control and improve through a healthy diet, be this in form of low-carb, high-fat or high-carb, moderate-fat.

The discussion surrounding carbohydrates does not simply stop at their potential impact on risk markers that we find in our blood. There is also some discussion whether carbohydrates or fats lead to an increase in waist circumference and the development of fat deposits in our bodies, whether carbohydrates or fats are the root cause of food cravings that we might experience and whether one or the other somehow helps us to move about more or makes us more sluggish.

As scientists and nutritionists we are naturally curious to find out what might be going on. So thank you for joining us!

## What to expect during you visits to our lab

There will be three appointments where we will ask you to come to IM Marsh campus (L17 6BD) for more thorough assessments, one right at the start when you will commence to eat according to the guidelines given to you, one after about 4 weeks and the final visit 8 weeks after your first one.

Each visit is expected to last between 60 and 90 minutes and will entail

**A venous blood sample**

We take about 8 teaspoons of blood. – Please note that you will have to have fasted for at least 12 hours prior to your appointment as otherwise your blood sugar and your triglycerides might be far higher than normal – painting a wrong picture of how the eating plan is working for you. Just as you had to do before coming in for your initial finger prick appointment you will also have to restrain from drinking alcohol or undertaking any strenuous exercise the night before. Again both can have an impact on your blood profile! We will analyse this blood sample at the end of the study to see how any risk factors for heart disease and type 2 diabetes might have changed over the course of eight weeks.

**Assessing your body composition**

This will be done in two different ways. Firstly, we will use a tape measure to measure your waist circumference, hip circumference, thigh circumference, calf circumference and neck circumference as these are all sites on the human body that can give us clues about the overall distribution of body fat. – Please ensure that you bring a pair of shorts with you to these visits as we will ask you to change into these before we take these measurements. If you prefer for a team member of the same sex to take these, please do let us know so that we can ensure that this can be facilitated.

Secondly, we will ask you to step onto sophisticated body composition scales (far bigger than the common bathroom ones) and measure your lean body mass, your body fat mass and the amount of fat surrounding your organs.

**Taking your blood pressure**

As blood pressure has been found to be an important factor in cardiometabolic health, we will assess your blood pressure every time you come to see us in our labs. Following standard protocol, we will take your blood pressure three times at each appointment and calculate the average of these three.

**Going through a brief questionnaire with you and conducting one final interview**

During your second and final visit we will go through a checklist to see which types of fibre-containing foods you have consumed over the previous four weeks.

During your final visit we will also ask you to stay with us for a little longer to conduct a brief interview with you asking you about your experiences with the diet allocated to you.

**Prior to your lab appointments – recording of physical activity**

On three occasions (just before your first, second and final visit to our lab) we will ask you to wear a physical activity monitor that looks like a digital watch and has to be worn on the wrist (just like a 'Fir bit') of your non-dominant arm. That means if you are right-handed you will have to wear this on your left wrist.

We will give you this device nine days before your visits to our labs and will ask you to start wearing this device for 7 days and at least 10 hours per day commencing on the morning after it has been handed to you and finishing the night before your lab appointment. During this time you will have to complete a wear-time diary on a daily basis, in which you will briefly record the times you are putting the monitor on in the morning, the times you are taking it off at night and any time during the time when you need to remove and put it back on, for example when you are taking a shower or when you are going swimming.

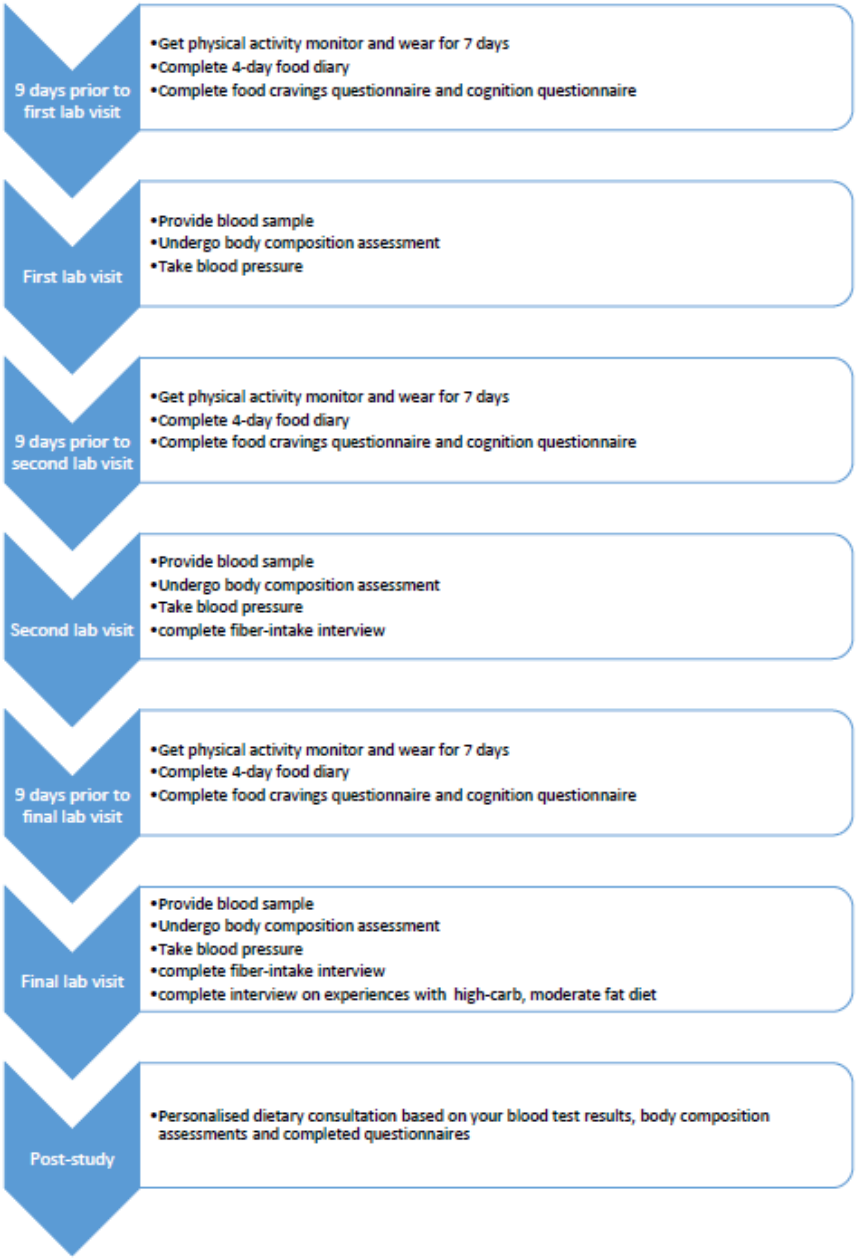
You will need to bring the monitor and the wear time diary with you on the morning of your lab appointment where the research team can collect it from you.

**Prior to your lab appointments – food diaries and questionnaires**

When you are given your physical activity devices we will also give you a template of a four-day food diary, which you will need to complete for four days before your lab appointment and bring with you on the morning.

We will also give you a number of brief questionnaires, which will assess your cognition and your food cravings over the previous four-week period. These will not take long to complete. Please also bring these questionnaires with you to your lab appointment and pass them on to our research team.

## Your involvement in the study – Flow chart high-carb group



## Following a high-carb diet – What does that actually mean?

First of all what a good time you have picked to join our study and follow a healthy, balanced diet. With summer just around the corner there is an abundance of foods that will make your eating delicious, interesting and affordable.

This guideline has been designed to help you follow the UK dietary recommendations over the next eight weeks whilst you are part of our cohort.

These recommendations are based around **starchy foods**, which means that at least **50%** of your diet should consist of carbohydrates. For the average man in the UK this means at least 333 grams per day, for the average woman in the UK at least 267 grams per day. (Women tend to be smaller than men and therefore require less energy and less food.) Due to the amount of carbohydrates recommended for consumption the UK recommended standard diet is classed as a high-carb diet.

These carbohydrates will come from a variety of sources, including potatoes, rice (brown is better!), pasta (wholemeal), bread (whole meal), fruit and vegetables and to a lesser extend dairy products (in the form of milk sugar, called lactose).

## Portion sizes for carbohydrates

So, what do at least 50% carbs look like?

Over the course of the day we should eat between 6 to 8 portions of starchy foods. If you are not a very active person (i.e. sit mainly at a desk in an office and only also do not move that much in your spare time) you should aim for 6 portions. If you are more active in your job, either your leisure time or both you can go for the 8 portions.

Try to consume about 2 portions at each main meal of the day.

| | |
|---|---|
| Breakfast cereal - e.g. bran flakes, cornflakes, rice crispies, porridge oats | 3 tablespoons (about 20g) |
| Shredded wheat | 1 biscuit |
| Weetabix | 1 biscuit |
| Muesli | 2 tablespoons |
| Bread | 1 slice (medium thick) |
| Bagel | Half |
| Bread roll | Half |
| Crackers | 3 |
| Crispbreads | 4 |
| Crumpet | 1 |
| Muffin | Half |
| Naan read, plain | 1 |
| Pitta bread | Half |

| | |
|---|---|
| Potatoes | 2 small boiled (egg-sized) |
| Jacket potatoes | Half (size of a computer mouse) |
| Rice, cooked | 2 heaped tablespoons (80g) |
| Noodles, boiled | 3 heaped tablespoons (80g) |
| Pasta, boiled | 3 heaped tablespoons (80g) |
| Malt loaf | 1 small slice |
| Muesli bar | 1 |

Here are some examples on how to achieve between 6 and 8 portions of starchy food per day:

| | Day 1 | Portions | Day 2 | Portions | Day 3 | Portions |
|---|---|---|---|---|---|---|
| **Breakfast** | 1 bowl of cereals (60g = 9 tablespoons) | 3 | 2 crumpets | 2 | 2 slices of toast | 2 |
| **Lunch** | Sandwich with two slices of bread | 2 | 1 filled pitta bread | 2 | Pasta salad (240g = 9 tablespoons of cooked pasta) | 3 |
| **Dinner** | Noodle stir-fry (160g = 6 heaped tablespoons of cooked noodles) | 2 | Rice with your meal (240g = 6 heaped tablespoons) | 3 | 4 small potatoes as side dish | 2 |
| **Total** | | 7 | | 7 | | 7 |

## Beware – Not all carbohydrates are equal!

This might actually be one of the issues with a high-carb diet, as with nearly everything in life, there is good and not so good, and the key is to eat more of the good stuff and hold back on the other stuff.

Many of the carbohydrates we consume in the Western world are highly processed – and the give-away is in the colour. Think white - so-called starchy foods – bread (flour), pasta, rice. Whilst it is okay to eat these occasionally, as adults we should focus on also incorporating wholemeal varieties into our daily eating. Maybe you are not used to eating these types and need some convincing? You could ease yourself into eating these by mixing for example white rice with whole-grain varieties or mixing different types of wholegrains. There are even some commercially available blends that can get you started.

The main reason for holding back on these white, processed varieties of starches is firstly that they are digested more easily by our bodies and reach our bloodstreams in the form of glucose a lot quicker. (These are also known as high-glycaemic index foods which you might have heard of before.) Secondly, the white colour is achieved by polishing the original grain and therefore removing a lot of vitamins and minerals that are a natural part of the food

matrix. In case you were wondering this is exactly the reason why wheat flour in the UK is fortified with a range of vitamins and minerals – the goodness that got taken out to begin with needs to be added again somehow.

When we are taking about wholegrain foods we do not only mean flour-based products. Other alternatives are naked barley, buckwheat, oats, quinoa, rye, teff and wild rice.

## What is fibre and why are we supposed to eat it?

Carbohydrates in our diet come from different sources, with some of them being more readily (if at all) absorbed by our bodies and used for energy to keep us going during the day. When nutritionists and other professionals talk about carbohydrates and a certain type of carbohydrate diet (in this case "high carbohydrate diet") we mean carbohydrates that are actually available to our bodies. Only these types of carbohydrates are actually being counted when making recommendations for carbohydrate intake. The other type of carbohydrates, which are generally not available, are classed as dietary fibre (see below). There are also different subcategories of these, with some supplying our bodies with small amounts of energy, but these can be disregarded in the context of the foods that we consume every single day as part of a healthy balanced diet.

The UK dietary recommendations state that everyone in the UK above the age of 14 should aim to consume at least 30 grams of fibre per day. At the moment the majority of the population is not meeting these recommendations. Research has shown that sufficient fibre intake can help prevent heart disease, some cancers and diabetes. Fibre can also aid to improve your digestion and make you feel fuller for longer meaning you eat less and less often (serial snackers beware). Fibre can be found in a number of starchy foods and fruit and vegetables, i.e. plant-based foods. This is also where wholegrain varieties of foods come into their own. They contain more fibre than their polished cousins.

Foods that contain 6 or more grams of fibre per 100g are classed as high-fibre foods, whereas those containing at least 3 grams of fibre per 100g are considered to be 'fibre-rich'.

Please note that if you think that your diet has been lacking in fibre so far to increase the amounts that you are consuming gradually over a couple of weeks and to ensure that you drink plenty of fluids. Otherwise you might have to deal with bloating and constipation as side effects.

### Foods containing fibre

Wholegrains (see list above), wholegrain cereals, bran cereals, wholegrain bread, brown rice, potatoes and oats are good sources of fibre. In addition, there is a wide variety of fruit and vegetables, and nuts and seeds that can be consumed to maintain or increase your fibre intake.

#### Fruit and vegetables (1 portion = 80g)

The table below shows the amount of fibre contained in one portion of 80g fruit or vegetable to give you an idea which foods might be good to prioritise in order to obtain sufficient fibre in your diet.

| Food name | Amount fibre in g (per 100g) | Amount fibre in g (per 80g portion) |
|---|---|---|
| Apples, eating, raw | 1.2 | 0.96 |
| Asparagus, boiled | 1.4 | 1.12 |
| Aubergines, fried | 2.3 | 1.84 |
| Avocado, average | 3.4 | 2.72 |
| Banana | 1.5 | 1.3 |
| Beans, baked in tomato sauce (tinned) | 3.8 | 3.04 |
| Beans, broad, boiled | 5.4 | 4.32 |
| Beans, butter, boiled | 5.2 | 4.16 |
| Beans, green, boiled | 2.5 | 2.0 |
| Beans, red kidney | 6.2 | 4.96 |
| Beans, soy/edamame | 6.1 | 4.88 |
| Beetroot, boiled | 1.9 | 1.52 |
| Blackberries | 3.1 | 2.48 |
| Broccoli, boiled | 2.3 | 1.84 |
| Broccoli, Purple sprouting, boiled | 2.3 | 1.84 |
| Brussel sprouts, boiled | 3.1 | 2.48 |
| Butternut squash | 1.4 | 1.12 |
| Cabbage, green cooked | 2.6 | 2.08 |
| Cabbage, red cooked | 2.0 | 1.6 |
| Cabbage, white cooked | 1.4 | 1.12 |
| Cabbage, spring greens, cooked | 2.6 | 2.08 |
| Carrots, young, boiled | 2.3 | 1.84 |
| Cauliflower, cooked | 1.6 | 1.28 |
| Celery, raw | 1.1 | 0.88 |
| Chickpeas, canned, re-heated | 4.1 | 3.28 |
| coconut, desiccated | 13.7 | 10.96 |
| Coconut, fresh | 7.3 | 5.84 |
| Courgette, boiled | 1.2 | 0.96 |
| Cucumber, raw | 0.7 | 0.56 |
| Fennel, Florence, boiled | 2.3 | 1.84 |
| Kale, curly, boiled | 2.8 | 2.24 |
| Kohlrabi, boiled | 1.9 | 1.52 |
| Leeks, boiled | 1.7 | 1.36 |
| Lentils, green or brown | 3.8 | 3.04 |
| Lentils, red | 1.9 | 1.52 |
| Lettuce, cos | 2.1 | 1.68 |

| | | |
|---|---|---|
| Lettuce, iceberg | 1.2 | 0.96 |
| Lettuce, romaine | 2.1 | 1.68 |
| Mushrooms, oyster, raw | 2.3 | 1.84 |
| Mushrooms, Portobello, grilled | 2.2 | 1.76 |
| Mushrooms, shitake | 2.1 | 1.68 |
| Mushrooms, white, boiled | 2.1 | 1.68 |
| Olives, green in brine, drained | 2.9 | 2.32 |
| Onions, fried (based on 2 table spoons, chopped) | 1.5 | 0.45 |
| Parsnip, boiled | 4.7 | 3.76 |
| Peanut butter, smooth | 5.4 | 1.62 |
| Peas, green, frozen, boiled | 4.0 | 3.2 |
| Pepper, green, boiled | 1.8 | 1.44 |
| Pepper, red, boiled | 0.8 | 0.64 |
| Pepper, yellow, boiled | 0.8 | 0.64 |
| Potatoes, boiled | 1.0 | 0.8 |
| Pumpkin, boiled | 1.1 | 0.88 |
| Radishes, raw | 0.9 | 0.72 |
| Raspberries, red | 2.5 | 2.0 |
| Rocket | 1.3 | 1.04 |
| Spinach, baby, raw | 1.2 | 0.96 |
| Spinach, frozen, boiled | 2.1 | 1.68 |
| Spring onion, raw | 1.5 | 1.2 |
| Strawberries | 1.0 | 0.8 |
| Sweetcorn kernels, canned in water, drained | 2.5 | 2.0 |
| Sweet potato, boiled | 2.3 | 1.84 |
| Tomato, raw | 1.0 | 0.8 |
| Turnip, boiled | 1.9 | 1.52 |
| Watercress | 1.5 | 1.2 |

### Nuts and seeds (1 portion = 30g)

A small handful (or about 30g) of nuts or seeds counts as a portion. Below some guidelines of how much fibre you would get from different types of nuts and seeds.

| Food name | Amount fibre in g (per 100g) | Amount fibre in g (per 30g portion) |
|---|---|---|
| Almonds, raw | 2.7 | 0.81 |
| Brazil nuts | 4.3 | 1.29 |

| | | |
|---|---|---|
| Chia seeds | 38 | 11.4 |
| Flaxseeds (Linseeds) | 27 | 8.1 |
| Hazelnuts | 6.5 | 1.95 |
| Peanuts | 6.2 | 1.86 |
| Peanut butter, smooth | 5.4 | 1.62 |
| Pecan nuts | 4.7 | 1.41 |
| Pistachios | 6.1 | 1.83 |
| Pumpkin seeds | 6.0 | 1.8 |
| Sunflower seeds | 6.0 | 1.8 |
| Walnuts | 3.5 | 1.05 |

## Sugar is a carbohydrate – right?

Yes, it is indeed! However, remember when we spoke about that some carbs are better (or worse) than others. Sugar in the form of free sugar should be kept to a minimum and again only be eaten occasionally as a treat. This means less than 31 grams per day for men (about 7 teaspoons) and less than 25 grams per day for women (about 6 teaspoons).

The trouble is that free sugar, which is the stuff that most people mean when they talk about sugar, does not simply come in the form of table sugar, honey or syrups where you control yourself how you add. It is actually hidden in many foods that we buy. Unsurprisingly the usual suspects are sweet-tasting foods such as chocolate, confectionary, cakes and sweetened and unsweetened fruit juices but the list of culprits also includes condiments (such as ketchup), crisps, bread, ready meals and canned goods, mayonnaise, the list goes on.

The best way to avoid free sugars is to do the majority of your cooking yourself and to become an avid food label-reader (and you don't even need a degree to crack this!)

## Eat a rainbow

You will have surely heard of the 5-a-day campaign, 5 portions of fruit and veg that is. There are actually several reasons why these are good for you. Firstly, they contain a considerable amount of carbs in the form of fibre – the good stuff. Secondly, the different colours express the presence of different types of nutrients that although not specifically named in the UK dietary guidelines (as there is actually thousands of them) that are really good for us and are thought to prevent heart disease, diabetes and cancers. These are namely anti-oxidants and polyphenols. The different colours of the fruit and vegetables available for us mean that these contain different types of these nutrients – all-round protection.

In general, it is recommendable to consume slightly more vegetables than fruit as these tend to contain more fibre and less fructose (fruit sugars).

If you feel slightly adverse to anything green and orange on your plate (one too many roast dinners with boiled-to-death cabbage) there are ways of incorporating these to help you feel less annoyed by their presence and to gradually get used to them. You could for example add

some finely chopped carrots and vegetables to a tomato sauce with your pasts. Alternatively, you could make a hummus with also contains vegetables such as beans, beetroot or peas to create a delicious snack.

## What exactly is a portion of fruit and veg?

A portion is defined as 80g of any fruit or vegetable and looks as follows:

### Fruit

#### Small fruit

| | |
|---|---|
| Apricots | 3 |
| Blackberries | 9 – 10 (One handful) |
| Blackcurrants | 4 heaped tablespoons |
| Blueberries | 4 heaped tablespoons (Two handfuls) |
| Cherries | 14 |
| Damsons | 5-6 |
| Figs, fresh | 2 |
| Fruit salad (fresh) | 3 heaped tablespoons |
| Grapes | 14 (One handful) |
| Kiwi | 2 |
| Kumquats | 6-8 |
| Lychee | 6 |
| Passion fruit | 5 - 6 |
| Plums | 2 |
| Raspberries | 20 (One handful) |
| Rhubarb, cooked | 2 heaped tablespoons |
| Satsumas | 2 |
| Strawberries | 7 |
| Tangerines | 2 |

#### Medium fruit

| | |
|---|---|
| Apple | One |
| Avocado | Half |
| Banana | One |
| Nectarine | One |
| Pear | One |
| Sharon fruit | One |

#### Large fruit

| | |
|---|---|
| Grapefruit | Half |
| Mango | Two slices (5cm/2 inches) |
| Melon | One slice (5cm/2 inches) |
| Papaya | One slice (5cm/2 inches) |
| Pineapple | One large slice |

### Dried fruit

30 grams of dried fruit count as one portion. Be careful with eating too much of these as the sugar in them is concentrated and can lead to tooth decay. It is better to incorporate these with other foods rather than eating on their own.

| | |
|---|---|
| Apple rings | 4 |
| Apricots | 3 |
| Banana chips | 1 handful |
| Cherries | 1 heaped tablespoon |
| Cranberries | 1 heaped tablespoon |
| Dates | 3 |
| Figs | 2 |
| Mango | 1 heaped tablespoon |
| Mixed fruit | 1 tablespoon |
| Peach | 2 halves |
| Pear | 2 halves |
| Pineapple | 1 heaped tablespoon or 2 rings |
| Prunes | 3 |
| Raisins, currants or sultanas | 1 heaped tablespoon |

### Fruit or vegetable juices

Note that only 150ml of any of vegetable or fruit juices or smoothies count towards one of your 5-a-day. This is because of the amount of free sugars (remember the 5% for sugar rule above!) contained in these juices. Try to choose unsweetened versions and limit consumption to meal times to prevent tooth decay.

### Tinned/canned/jarred fruit

Some of these also contain a lot of sugar if syrup rather than water is used to contain the fruit. Check the label to be aware how much added sugar you might be consuming in this case!

| | |
|---|---|
| Apples | 2 heaped tablespoons |
| Apricots | 6 halves |
| Cherries | 11 (Three heaped tablespoons) |
| Fruit salad | 3 heaped tablespoons |
| Grapefruit segments | 3 heaped tablespoons or 8 segments |
| Lychee | 6 |
| Mandarin oranges | 3 heaped tablespoons |
| Peaches | 2 halves or 7 slices |
| Pear | 2 halves or 7 slices |
| Pineapple | 2 rings or 7 chunks |
| Prunes | 6 |
| Raspberries | 20 (One handful) |
| Rhubarb, cooked | 5 chunks |
| Strawberries | 9 |

## Vegetables

### Fresh, frozen or cooked vegetables

| | |
|---|---|
| Artichoke hearts | 2 |
| Asparagus | 5 spears |
| Aubergine | 1/3 |
| Beetroot | 3 whole baby beetroot or 7 slices |
| Beans, French or runner | 4 heaped tablespoons |
| Broccoli | 2 spears or 8 florets |
| Brussel sprouts | 6-8 |
| Butternut squash | 3 heaped tablespoons |
| Cabbage, cooked | 4 heaped tablespoons |
| Cabbage, shredded | 3 heaped tablespoons |
| Carrot, sliced | 3 heaped tablespoons |
| Carrot, shredded | 3 heaped tablespoons |
| Cauliflower | 8 florets |
| Chinese leaves or Pak Choi, shredded | 4 heaped tablespoons |
| Corn in the cob | 1 |
| Leek, medium-sized (white portion only) | 1 |
| Mange-tout | 1 handful (about 22) |
| Marrow, diced | 3 heaped tablespoons |
| Mixed frozen vegetables | 3 heaped tablespoons |
| Mushrooms, sliced | 3-4 heaped tablespoons |
| Mushrooms, button | 14 |
| Okra, medium | 9 |
| Onion | 1 |
| Parsnip, medium | 1 |
| Peas | 3 heaped tablespoons |
| Pepper | half |
| Pumpkin | 3 heaped tablespoons |
| Spinach, cooked | 4 heaped tablespoons |
| Spring greens, cooked | 4 heaped tablespoons |
| Spring onions | 8 |
| Swede | 3 heaped tablespoons |
| Sweetcorn, baby | 6-8 |
| Sweet potato, medium | 1 |
| Tomato puree (Yes, technically a fruit) | 1 heaped tablespoon |
| Turnip, diced | 3 heaped tablespoons |

### Salad vegetables

| | |
|---|---|
| Celery stick | 3 |
| Cucumber | 5 cm/2-inch piece |
| Lettuce or mixed leaves | 1 cereal/dessert bowl |
| Radishes | 10 |
| Rocket | 1 cereal/dessert bowl |

| | |
|---|---|
| Spinach, fresh | 1 cereal/dessert bowl |
| Tomato, cherry | 7 |
| Tomato, medium | 1 |
| Watercress, fresh | 1 cereal/dessert bowl |

### Tinned/canned/dried

Try and choose brands that are low in added salt and sugar (read the label).

| | |
|---|---|
| Asparagus | 7 spears |
| Beetroot | 3 whole baby or 7 slices |
| Carrots, canned | 3 heaped tablespoons |
| Mushrooms, dried | 2 tablespoons |
| Peas | 3 heaped tablespoons |
| Sun-dried tomatoes | 4 |
| Sweetcorn | 3 heaped tablespoons |
| Plum tomatoes | 2 |

### Pulses (beans and lentils)

No matter how many portions you eat over the course of the day due to their high protein content only one portion of beans or lentils (pulses) will be counted as your 5-a-day.

Pulses include cooked baked beans, borlotti, black eye, broad, butter, cannellini, kidney, pinto, soy beans, chickpeas, green, yellow, red or black lentils. One portion is 3 heaped tablespoons.

### Potatoes

These do not count as one of your 5-a-day as they are already part of the starchy portion of the Eatwell Guide, the UK dietary guidelines.

## Dietary fat – the evil nutrient? Following a moderate-fat diet

No, not quite! The UK dietary guidelines actually de facto recommend a moderate-fat diet of less than 35%, which is about 97 grams per day for the average UK man and 78 grams per day for the average UK woman aged 11-64. One tablespoon of oil equals 15 grams, this means that the UK average man should consume no more than 6 ½ tablespoons of fat per day, the average UK woman no more than about 5 tablespoons. It is important to note that the majority of fat we consume will not be visible to the eye but incorporated into foods and dishes that we buy and eat.



Although it is recommended to stick to low-fat products overall, fat does play an important role in our diet. It helps to absorb some vitamins that would otherwise simply go right through us. It also plays an important role in building the membranes of our cells. The key lies in the

quality of the fats consumed with priority given to unsaturated fats, such as olive oil and other vegetable oils, avocados, nuts and oily fish. One type of unsaturated fat which is essential to human health are omega-3 fatty acids. These can be found both in animal and plant sources. Oily fish (see below) is an important source of omega-3 fatty acids as these are of the highest quality. However, it is possible for the body to convert the omega-3 fatty acids found in plants foods, such as nuts and seeds and their products, into the same end products found in oily fish.

Men should consume no more than 30g (about 2 tablespoons) and women no more than 20g (about 1 ½ tablespoons) of saturated fats, which can be found in chocolate, pastries, cakes and biscuits, dairy products (such as full-fat milk, cream, butter) and fatty cuts of meat and sausages. This means to go easy on the butter and use unsaturated fat spreads instead, use lower-fat milk (green top or red top for example) and only have that hot chocolate with lots of whipped cream on top only as the occasional treat. The same goes for cakes, biscuits, crisps and other snack foods. This means being careful with the snacks that you choose over the course of the day should you get a bit peckish.

When looking for products that are lower in fat it is important to know that there is a difference between 'low-fat' and 'reduced fat' products. The first category means that the food contains 3g or less fat per 100g whereas a reduced fat food contains 25% less fat than the standard product. This might mean that even the reformulated product is still fairly high in fat.

## Proteins to help you build and maintain that temple which is your body

Proteins are important in our diet as they have the vital functions of growth, maintenance and repair. They are also vital to help our immune system function properly. Proteins are composed of compounds called *amino acids* of which there are 20 that play a role in the human body. 8 of these amino acids are classed as essential because unlike the other 12 our bodies cannot produce these themselves, which means that they need to be obtained from our diets. This happens by consuming protein foods.

Whilst protein malnutrition can have disastrous consequences in reality the vast majority of people in the UK consume more than they actually need to.

The average UK male aged 15 to 64 should consume about 55 grams per day, the average UK female in this age group 45 grams per day. In order to achieve these recommendations we should eat two to three portions of protein each day and another two to three portions of dairy. There are different types of protein sources, including:

### Animal-based proteins

Animal-based proteins contain all of the essential amino acids. This is because the animals have done the work for us eating a variety of food sources with different amino acid profiles

and combing these in their muscles. The same is true for eggs where the egg yolk and the egg white have the role of sustaining the developing chick.

*Meat*

Aim for lean cuts of meat and lean meat mince as meat can be high in saturated fatty acids. For the same reason it is advisable to cut off the visible fat from beef and pork steaks and to remove the skin from chicken. The way you cook your meat also plays an important role in your diet. Frying meats can add to your fat intake, so it is better to grill your meat instead, to use non-stick frying pans or to use spray bottles for oil, which will help you to avoid unnecessary fat to your pan. Other lower-fat methods include boiling and steaming.

You should not eat more than 70g of processed meat per day, which includes sausages, bacon, cured meats (for example salami, chorizo) and reformed meats products (for example sliced packaged ham).

*Fish and seafood (Shellfish)*

You should eat at least two portions of fish per week, one white, one oily. One portion is 140 grams which is about the size of a cheque book. The reason we recommend oily fish because these are an excellent source of omega-3 essential fatty acids, which we have mentioned earlier on. Fish also supply us with a number of vitamins and minerals.

**White fish**

| | | | | | |
|---|---|---|---|---|---|
| Basra | Cod, | Coley | Dab | Flounder | Gurnard |
| Hake | Haddock | Plaice | Pollock | Red mullet | Tilapia |

Some white fish should be eaten no more than once a week due to potentially high levels of pollutants contained in their flesh. These are seabeam, seabass, halibut and turbot.

**Oily fish**

| | | | | | |
|---|---|---|---|---|---|
| Anchovies | Carp | Herring | Kippers | Mackerel | Pilchard |
| Salmon | Sardines | Sprats | Trout | Tuna (fresh or frozen) | Whitebait |

Swordfish should not be eaten more than once a week due to potentially high levels of pollutants. It should be avoided by children, pregnant women and those wanting to become pregnant.

**Shellfish**

| | | | |
|---|---|---|---|
| Cockles | Crab | Langoustines | Mussels (and clams and winkles) |
| Oysters | Prawns | Scallops | Squid |

It is important to note that processed, canned fish like tuna do no longer count as your 'oily' portion as the manufacturing process has taken all the omega-3 fatty acids out of them.

However, the good news is – that tuna mayo sandwich for your lunch still counts towards your portion of white fish.

One thing to bear in mind is choosing where possible sustainable sources of fish as recommended by the Marine Stewardship Council (MSC) in their *Good Fish Guide*. The MSC also run an accreditation scheme, so look out for certified products carrying the following logo:

Further information on the MSC and a regularly updated list of sustainable types of fish can be found at www.msc.org.

*Eggs*

Eggs are real powerhouses of nutrition and contain all the essential amino acids that humans need in their diet. One medium egg provides about 6.4grams of protein with the egg yolk containing higher concentrations of amino acids. However, as the egg white is larger than the egg yolk the majority of protein supplied when eating an egg will actually come from the egg white.

For animal welfare reasons we would recommend that you avoid buying the eggs from caged hens. However, the choice is absolutely yours.

*Dairy*

Dairy products are both a source of protein and fats. Aim therefore for low-fat versions of dairy where possible. Dairy is also a good source of calcium and should therefore be included in our diet on a daily basis. It is recommended that we consume 2 to 3 portions of dairy (or plant-based, calcium-fortified alternatives) per day in addition to the other protein sources.

Dairy products include milk, cream, yoghurt, cheese, cream cheese, sour cream and quark. If you dislike dairy products, it is recommended to use calcium-fortified plant milks instead, such as soya, rice, oat and almond milks.

Plant-based proteins

These include beans, peas, lentils and chickpeas, which are high in fibre, vitamins and minerals and low in fat.

Nuts and seeds are also a great source of proteins but can contain a lot of fat. However, these are great sources of unsaturated fats, included important omega-3s.

Other plant-based protein sources include tofu, bean curd, mycoprotein (such as Quorn™) and soya-based meat replacement products, such as supermarkets' own brands. Please remember that the latter two are processed foods so it is always worth checking the label to ensure that there are not too many additives and flavour enhancers, salt and sugar found in these products. If this is the case, you should either eat these products sparingly or ensure that you count these towards your salt and sugar consumption for the day.

The proteins found in plants do normally not contain all of the essential amino acids (a more widely consumed exception are soya beans). This means that a combination of plant foods needs to be consumed to achieve a complete profile. Examples of this are beans on toast (i.e.

beans and flour), rice and beans, rice and lentils, crostini/melba toast with broad bean spread. Combing a cereal with a pulse in general will achieve a complete amino acid profile.

## Portion sizes for protein

| | |
|---|---|
| Meat, cooked | 80g (size of a deck of cards or 3 slices) |
| Fish | 140g (size of a cheque book) or 3 fish fingers |
| Eggs | 2 medium-sized |
| Milk | 200ml (medium glass) |
| Plant-based milks, such as soya, rice, almond, oat – fortified with calcium | 250ml (large glass) |
| Yoghurt | 125g (small container) |
| Hard cheese | 40g (matchbox-size piece) or 40g (2 slices) |
| Cottage cheese | 200g (large pot) |
| Cream cheese, light | 80g (about 2 small matchboxes) |
| Fromage frais | 150g (small container) |
| Sour cream | 2 tablespoons |
| Baked beans | 200g (small tin) |
| Pulses, cooked | 4-5 tablespoons |
| Nuts and seeds | 40g (1 small handful) |
| Nut butters, such as peanut butter | 2 tablespoons (about the size of a golf ball) |
| Meat-replacement products | 120g (for example two sausages or about the size of a snooker ball) |

## Treats or foods full of bliss – AKA Foods high in fats, salts and sugars

These are foods that are in general of no nutritional value to our bodies and in consumed in excess can do more harm than good, for example chocolate, cakes, biscuits, crisps, soft drinks, ice-cream, processed pork pies and sausage rolls. Remember the rules not to consume more than 7 teaspoons of sugar and 20 to 30 grams of saturated fats per day? The foods in the *treats* category might jeopardise this goal. If you find yourself eating these things to reward yourself, because you are having a bad day or simply because they are there – talk to us so that we can try and help you to find ways of limiting their consumption.

One of these ways might include to really sit down with a cup of coffee and that doughnut or slice of cake and take your time enjoying it rather than eating mindlessly. Taking your time and appreciating this type of food will also allow your digestive system enough time to connect with your brain and consequently to signal your stomach that you are full and tell your mind that you are happy.

## Hydration

This is an important one that often gets overlooked. You should drink 6-8 glasses (200ml or medium-sized) per day to ensure staying hydrated. This will also help your body to cope with a potential increase in dietary fibre following a healthy diet (see above). The UK dietary

recommendations advise that you best stick to water, unsweetened teas and coffee and lower-fat milk.

Limit intake of fruit juices and smoothies (only 150ml per day count as one of your five-a-day) as the natural sugar contained in fruits has been freed through the process of liquidisation. This means it is more easily digested and can raise your blood sugar a lot quicker. The fruit sugar contained in fruit (fructose) can actually not be metabolised by our bodies and if not turned into energy will be converted into body fat. In addition, this free sugar can contribute to dental decay.

Eating whole fruit is not so much of a problem as the sugar is contained within a matrix plus whole fruit has the added benefit of fibre (see above).

That can of coca cola that you might enjoy at lunchtime should become an occasional treat only (see above). Soft drinks contain up to nearly 16 grams (or nearly four teaspoons) of sugar per 100ml, which means you will go over the recommended daily intake very quickly.

## Learning to read labels and nutritional information

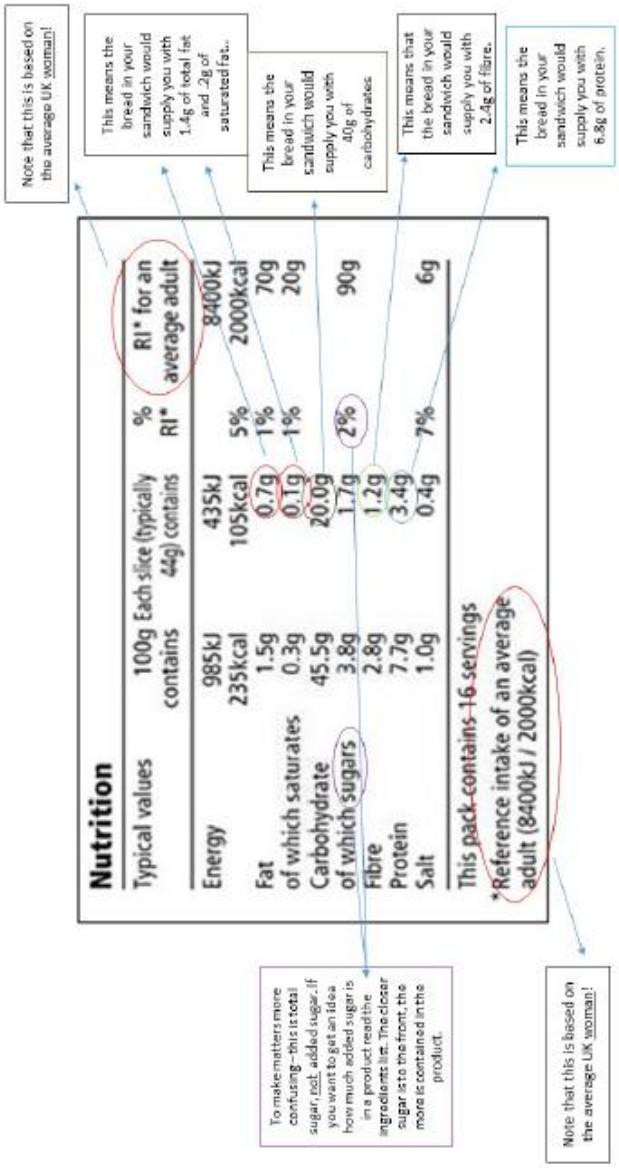
This is an important one when food shopping and eating out in places that provide you with information on the nutritional content of their dishes, for example fast food restaurants and pubs.

### Label reading

In the UK food labels can be found at the back and depending on the manufacturer also at the front of the packaging.

Labels at the back of the packaging

Below is the back of pack nutrition label for a loaf of white bread as an example.



There are a few things to be aware of:

The manufacturer's portion size guide might actually not be a realistic reflection of what you might actually eat yourself and it can be very easy to underestimate the amount of sugars and fats that you are consuming.

Whilst it is fairly easy to work out how many carbohydrates you might consume over the course of a day using food label, calculating how much free sugars (remember, the 5% rule!) is an entirely different matter. The label will not tell you these things and total sugars will be a combination of the natural sugars contained in foods and can also include the sugars found in fruits (fructose) and milk (lactose) where applicable.

This is where looking at the ingredients list (also a the back of pack) might necessary as sugar comes under many different names and there might be some surprises in store for you.

As a general rule, the closer an added sugar is towards the top of the ingredients list the more of it will be contained in the product. There might also be different types of added sugar in the same product.

All of these that you might spot in the ingredients list are actually added sugars

> agave sugar, brown sugar, cane sugar, dextrose, fructose, fruit juice concentrate, glucose, HFCS/high fructose corn syrup, honey, hydrolysed starch, invert sugar, isoglucose, levulose, maltose, modified starch molasses, sucrose, syrups (sucrose, glucose, malt, corn, maple), treacle,

Once a product crosses specific thresholds for specific nutrients it will either be classed as 'medium' or 'high' in sugar or other nutrients (see below).

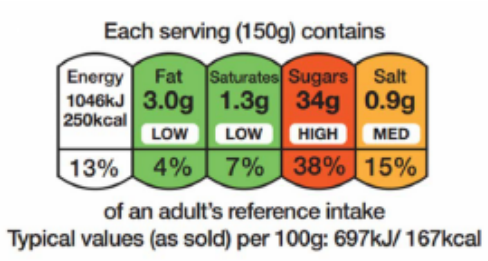
| | Low | Medium | High |
|---|---|---|---|
| **Total sugars** | 5g per 100g product (or 5%) | More than 5g but less than 22.5 per 100g product (between 5% and 22.5%) | More than 22.5g per 100g product (or 22.5%) |
| **Total fats** | 3g per 100g product (or 3%) | More than 3g but less than 17.5 per 100g product (between 3% and 17.5%) | More than 17.5g per 100g product (or 17.5%) |
| **Saturated fats** | 1.5g per 100g product (or 1.5%) | More than 1.5g but less than 5g per 100g product (between 1.5% and 5%) | More than 5g per 100g product (or 5%) |
| **Salt** | 0.3g of salt or less per 100g (or 0.1g sodium) | More than 0.3g of salt (0.1g sodium) but less than 1.5g of salt (0.6g sodium) per 100g product | More than 1.5g of salt per 100g (or 0.6g sodium) |

If the portion size means that more than 25g of sugar would be consumed with one portion the product is automatically classed as 'high sugar'.

For drinks containing total sugar the rule is that 2.5g per 100ml is classed as 'low', between 2.5 and 11.25g per 100ml is classed as 'medium' and above 11.25g per 100ml is 'high'. If the portion size of a drink means that more 13.5g of sugar would be consumed the product is automatically classed as 'high sugar'.

### Front of pack labels

Some retailers and manufacturers display a food label at the front of the pack highlighting whether some of the crucial nutrients are contained in the foods to low, medium or high levels. This is called the 'traffic light system'.



### Interpreting nutritional information provided by fast food restaurant and pub chains

A number of high street fast food outlets and pub chains publish nutritional information on typical serving sizes of their dishes that you can access online, download or on request at the restaurant.

Being aware of the nutritional content of some of these dishes is helpful if you tend to eat out more often as it can be difficult in this case to stick to a healthy, balanced diet and you might unknowingly jeopardise this balance.

It is also great to make you aware of how much individual components will add up. A lot of menus will inform you about the extra calories that adding chips or a jacket potato will add to your dish. However, they fail to explain where exactly these calories are coming from. A good example is choosing a chicken burger thinking that the saturated fat content of these won't be too bad but adding extra cheese and bacon might add more saturated fats than you think. The same goes for building that perfect sandwich. Even more care has to be taken with hidden fats and added sugars in salad dressings.

However, if you do not eat out very often and are overall doing fine following the guidelines, then by all means make this your occasional treat!

Some example nutrition guides are attached to this guide to give you an idea what to look out for. However, these are by no means meant as endorsements of particular eateries.

## Your appointment schedule

| Physical activity monitor collection//delivery | First lab visit | Physical activity monitor collection/ /delivery | Second lab visit | Physical activity monitor collection/ /delivery | Third lab visit |
|---|---|---|---|---|---|
| | | | | | |

All lab visits will take place at LJMU IM Marsh campus in Mossley Hill (L17 6BD).

## Your contacts

Your main contact is Tanja Harrison who is also a registered associate nutritionist with the Association for Nutrition.

M 07970 858 594

E T.harrison@2015.ljmu.ac.uk

If you have any queries or concerns throughout the study, please do not hesitate to contact Tanja.

Other researchers on the team that you will meet on a regular basis

Deaglan McCullough

E d.mcullough@2015.ljmu.ac.uk

Dr Ian Davies (A registered nutritionist, focus on nutrition science)

E i.g.davies@ljmu.ac.uk

Dr Katie Lane (A registered nutritionist, focus on food)

E k.e.lane@ljmu.ac.uk

Dr Kevin Enright

E k.j.enright@ljmu.ac.uk

This study was approved by LJMU's Research Ethics Committee on 16 December 2016 (Ref. 16/ELS/029). If you any concerns regarding your involvement in this research, please discuss these with the researcher in the first instance. If you wish to make a complaint, please contact researchethics@ljmu.ac.uk and your communication will be re-directed to an independent person as appropriate.

## Further information throughout the study

You can also find all the materials in a private Facebook Group, which you can request to join should you wish to do so at https://www.facebook.com/groups/CALIBERhighcarb/

We will also post regular updates here. However, should you not wish to join all the links and information will be provided to you via email or in hard copy.

## Appendix - Helpful App to help you following the official UK dietary recommendations

### Change4Life Be Foodsmart App


Change4Life is a public health initiative run by Public Health England. This app lets you scan the barcodes of about 130,000 food products available from UK major manufacturers and retailers. It focuses on the amount of sugar, salt and saturated fats in these foods and uses a traffic light system to let you know whether these would be high in the nutrients.

The app can be downloaded for free from the Appstore and GooglePlay.

### Change4Life – Sugar smart App


This app lets you scan the barcodes of about 87,000 food products available from UK major manufacturers and retailers. It focuses on the amount of free sugar in these foods and uses a traffic light system to let you know whether these would be high in the nutrients.

The app can be downloaded for free from the Appstore and GooglePlay.

## Appendix - Examples nutritional information fast food, pub and restaurant chains

### Brewers Fayre

| Product/ Dish Description | Nutrition Information Per Portion | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | kJ | kcal | Fat (g) | Saturates (g) | Carbohydrate (g) | Sugars (g) | Protein (g) | Salt (g) |
| **DAYTIME VALUE & SNACKS MENU** | | | | | | | | |
| **STARTERS** | | | | | | | | |
| TOMATO SOUP | 980 | 234 | 7.7 | 3.3 | 33.0 | 11.8 | 5.7 | 1.8 |
| CRISPY POTATO DIPPERS | 2045 | 488 | 28.6 | 12.1 | 34.0 | 2.7 | 23.6 | 1.7 |
| BUBBLE & SQUEAK | 1458 | 348 | 24.4 | 10.9 | 19.5 | 3.4 | 10.4 | 1.4 |
| GARLIC & HERB BREADED MUSHROOMS | 1627 | 389 | 17.3 | 1.9 | 53.0 | 6.7 | 8.7 | 1.5 |
| **MAINS** | | | | | | | | |
| SMOTHERED CHICKEN | 3991 | 953 | 45.3 | 15.1 | 79.0 | 11.9 | 55.4 | 4.5 |
| LASAGNE | 2653 | 634 | 28.1 | 12.1 | 64.3 | 17.0 | 27.9 | 3.1 |
| GRILLED GAMMON STEAK WITH EGGS | 3841 | 917 | 37.8 | 12.5 | 62.5 | 5.6 | 80.1 | 4.5 |
| GRILLED GAMMON STEAK WITH PINEAPPLE | 3655 | 873 | 31.3 | 10.8 | 81.2 | 26.4 | 67.8 | 4.2 |
| GRILLED GAMMON STEAK WITH ONE OF EACH | 3748 | 895 | 34.6 | 11.6 | 71.9 | 16.0 | 74.0 | 4.3 |
| FISH & CHIPS WITH PEAS | 5227 | 1248 | 73.2 | 12.3 | 107.4 | 6.9 | 37.5 | 1.7 |
| FISH & CHIPS WITH MUSHY PEAS | 5386 | 1286 | 73.3 | 12.4 | 113.9 | 5.6 | 39.8 | 2.6 |
| BATTERED GIANT HADDOCK & CHIPS WITH PEAS | 4501 | 1075 | 59.3 | 16.2 | 86.3 | 7.7 | 47.4 | 1.7 |
| BATTERED GIANT HADDOCK & CHIPS WITH MUSHY PEAS | 4660 | 1113 | 59.4 | 16.3 | 92.8 | 6.4 | 49.7 | 2.6 |
| MEXICAN BEEF CHILLI | 2999 | 716 | 18.6 | 5.4 | 97.1 | 5.7 | 28.7 | 2.2 |
| THREE CHEESE CRUSTLESS QUICHE | 2390 | 571 | 36.4 | 18.0 | 42.2 | 10.6 | 19.7 | 1.2 |
| THE SOUTH WESTERN BURGER | 4105 | 980 | 47.6 | 11.0 | 106.5 | 12.4 | 29.6 | 2.4 |
| BEEF, CHEESE & MUSHROOM BURGER | 4846 | 1157 | 64.5 | 23.0 | 93.3 | 12.3 | 48.7 | 2.5 |
| GRILLED CHICKEN & BACON SALAD | 1816 | 434 | 19.0 | 5.6 | 12.4 | 11.3 | 50.9 | 4.7 |
| CHICKEN TIKKA CURRY | 3614 | 863 | 24.0 | 6.2 | 113.1 | 25.6 | 45.3 | 3.7 |
| BREADED WHOLETAIL SCAMPI WITH PEAS | 3885 | 928 | 44.7 | 8.3 | 107.0 | 7.0 | 24.2 | 3.9 |
| BREADED WHOLETAIL SCAMPI WITH MUSHY PEAS | 4044 | 966 | 44.8 | 8.4 | 113.6 | 5.7 | 26.5 | 4.8 |
| SMOKY PAPRIKA CHICKEN | 1802 | 430 | 14.7 | 4.1 | 31.9 | 13.1 | 41.4 | 2.2 |
| SAUSAGE, EGG & CHIPS | 4170 | 996 | 57.1 | 18.9 | 74.2 | 7.4 | 44.0 | 2.5 |
| SWEET POTATO & FETA LASAGNE | 3106 | 742 | 39.1 | 15.8 | 69.9 | 17.8 | 24.2 | 3.0 |
| HOT 'N' SPICY VEGGIE NACHO BURGER | 5160 | 1233 | 61.8 | 19.9 | 142.3 | 17.9 | 23.0 | 3.0 |
| HAM & CHEESE SANDWICH WITH WHITE BREAD | 2613 | 624 | 28.3 | 12.6 | 55.8 | 3.0 | 35.2 | 3.5 |

## Harvester

| STARTERS AND SHARERS | Energy (KJ) | Energy (Kcal) | Fat (g) | Saturated Fat (g) | Carbohydrate (g) | Sugars (g) | Protein (g) | Salt (g) |
|---|---|---|---|---|---|---|---|---|
| Potato Skins with Bacon & Cheese | 1,934 | 460 | 26.2 | 13.5 | 32.2 | 2.1 | 22.8 | 2.15 |
| BBQ Chicken Wings | 1,593 | 379 | 18.4 | 4.8 | 20.3 | 18.6 | 32.8 | 1.98 |
| Crispy Coated Chicken Bites | 1,683 | 401 | 17.9 | 3.2 | 36.9 | 18.3 | 22.9 | 2.21 |
| Cheese Popits with Salsa V | 1,759 | 419 | 25.9 | 10.1 | 33.1 | 4.4 | 12.2 | 1.46 |
| Spicy Crackerjack King Prawns | 1,358 | 324 | 13.3 | 3.1 | 40.0 | 13.6 | 9.3 | 2.02 |
| Breaded Mushrooms V | 2,363 | 563 | 39.2 | 6.0 | 43.6 | 2.2 | 8.1 | 1.88 |
| Chicken & Chorizo skewers NEW | 1,990 | 474 | 36.5 | 12.4 | 6.7 | 5.5 | 28.3 | 1.81 |
| Ultimate Nachos V | 3,633 | 865 | 48.7 | 15.8 | 82.0 | 4.8 | 20.3 | 3.03 |
| *Why not add Three Bean Chilli V* | 464 | 110 | 3.0 | 0.4 | 15.1 | 6.8 | 4.2 | 0.78 |
| *Or add BBQ Pulled Pork* | 1,298 | 309 | 10.1 | 3.3 | 40.4 | 38.7 | 13.6 | 1.43 |
| Creamy Tomato & Basil Soup V NEW | 1,268 | 302 | 5.7 | 2.7 | 53.5 | 4.9 | 8.0 | 2.26 |
| Sticky Duck Wings | 1,839 | 438 | 19.1 | 5.1 | 35.5 | 32.5 | 29.2 | 2.69 |
| Fish Basket NEW | 2,994 | 713 | 45.2 | 7.5 | 54.5 | 7.5 | 21.7 | 4.00 |
| Cheesy Garlic Bread Board V NEW | 5,795 | 1,380 | 92.6 | 31.8 | 108.4 | 15.3 | 34.0 | 5.15 |
| **CHICKEN** | **Energy (KJ)** | **Energy (Kcal)** | **Fat (g)** | **Saturated Fat (g)** | **Carbohydrate (g)** | **Sugars (g)** | **Protein (g)** | **Salt (g)** |
| Harvester's Famous 1/2 Rotisserie Chicken | 1,885 | 449 | 22.9 | 5.0 | 2.4 | 3.5 | 57.1 | 1.58 |
| Whole Rotisserie Chicken NEW | 3,525 | 839 | 41.2 | 10.0 | 2.4 | 4.0 | 113.5 | 3.07 |
| Chargrilled Chicken Breast | 1,126 | 268 | 9.1 | 1.0 | 2.5 | 3.1 | 43.0 | 1.48 |
| Triple Chicken | 2,763 | 658 | 27.7 | 5.7 | 13.6 | 3.6 | 87.3 | 2.94 |
| Chicken Skewer | 1,879 | 447 | 25.9 | 6.0 | 2.5 | 3.3 | 49.7 | 0.92 |
| Salsa Chicken & Pepper Stack | 1,588 | 378 | 14.0 | 1.2 | 11.4 | 11.4 | 49.0 | 1.90 |
| HARVESTER RECOMMENDS BBQ Brushed & Basted | 4,487 | 1,068 | 43.8 | 9.3 | 103.9 | 47.4 | 63.0 | 3.67 |
| HARVESTER RECOMMENDS Piri Piri Brushed & Basted | 3,952 | 941 | 48.0 | 9.6 | 63.8 | 8.0 | 62.1 | 6.22 |
| Garlic & Parsley Brushed & Basted | 6,259 | 1,490 | 40.3 | 12.9 | 59.9 | 73.1 | 69.8 | 3.1 |
| Farmer Rikki's Hot Chilli Sauce Brushed & Basted | 4,117 | 980 | 52.0 | 9.9 | 62.7 | 5.8 | 63.1 | 2.99 |

## Pizza Hut

| | Pizza Weight (g) | Number of Slices per Pizza | Energy per Slice (kcal) | Protein per Slice (g) | Carbohydrate per Slice (g) | Sugar per Slice (g) | Fat per Slice (g) | Saturated Fat per Slice (g) | Salt per Slice (g) | Energy per 100g (kcal) | Protein per 100g (g) | Carbohydrate per 100g (g) | Sugar per 100g (g) | Fat per 100g (g) | Saturated Fat per 100g (g) | Salt per 100g (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Triple Pepperoni** | | | | | | | | | | | | | | | | |
| Individual Pan (9") | 459 | 6 | 228 | 9.2 | 20.5 | 1.4 | 12.0 | 4.0 | 1.08 | 298 | 12.0 | 26.8 | 1.8 | 15.7 | 5.3 | 1.42 |
| Sharing Pan (13") | 954 | 8 | 353 | 11.2 | 32.6 | 2.1 | 18.1 | 6.1 | 1.70 | 296 | 12.0 | 27.3 | 1.8 | 15.2 | 5.1 | 1.42 |
| Individual Thin (11") | 445 | 6 | 188 | 7.5 | 18.7 | 0.9 | 8.9 | 3.5 | 0.71 | 253 | 12.7 | 25.2 | 1.2 | 12.0 | 4.7 | 0.95 |
| Sharing Thin (14") | 768 | 8 | 258 | 9.7 | 21.3 | 1.4 | 14.0 | 6.1 | 1.58 | 269 | 12.7 | 22.2 | 1.5 | 14.6 | 6.4 | 1.64 |
| Individual Stuffed Crust (11") | 561.5 | 6 | 259 | 11.2 | 19.9 | 0.9 | 13.8 | 6.5 | 1.48 | 277 | 14.1 | 21.2 | 0.9 | 14.7 | 6.9 | 1.58 |
| Sharing Stuffed Crust (14") | 762 | 8 | 279 | 14.9 | 25.4 | 1.4 | 14.3 | 4.5 | 1.34 | 293 | 12.6 | 26.6 | 1.5 | 15.0 | 4.7 | 1.41 |
| Cheesy Bites (14") | 762 | 8 | 279 | 14.9 | 25.5 | 1.4 | 14.5 | 4.6 | 1.34 | 293 | 12.6 | 26.8 | 1.5 | 15.2 | 4.8 | 1.41 |
| Gluten Free (9" Square) | 456 | 6 | 209 | 4.8 | 20.8 | 1.3 | 10.3 | 3.7 | 1.19 | 275 | 8.9 | 27.4 | 1.7 | 13.6 | 4.8 | 1.57 |
| **Meat Feast** | | | | | | | | | | | | | | | | |
| Individual Pan (9") | 475 | 6 | 203 | 9.6 | 20.8 | 1.5 | 8.8 | 3.0 | 0.91 | 256 | 12.2 | 26.2 | 1.9 | 11.2 | 3.7 | 1.15 |
| Sharing Pan (13") | 1001 | 8 | 327 | 15.8 | 33.0 | 2.4 | 14.3 | 4.8 | 1.49 | 261 | 12.7 | 26.4 | 1.9 | 11.4 | 3.8 | 1.19 |
| Individual Thin (11") | 474 | 6 | 178 | 10.0 | 20.1 | 1.0 | 6.1 | 2.5 | 1.02 | 226 | 12.6 | 25.4 | 1.3 | 7.8 | 3.2 | 1.29 |
| Sharing Thin (14") | 829 | 8 | 242 | 14.4 | 22.8 | 1.5 | 9.9 | 4.0 | 1.43 | 234 | 13.9 | 22.0 | 1.5 | 9.6 | 3.9 | 1.38 |
| Individual Stuffed Crust (11") | 578 | 6 | 233 | 13.5 | 20.2 | 1.1 | 10.6 | 5.4 | 1.28 | 242 | 14.0 | 21.0 | 1.1 | 11.0 | 5.6 | 1.33 |
| Sharing Stuffed Crust (14") | 1107 | 8 | 336 | 19.6 | 30.2 | 1.6 | 14.7 | 7.0 | 1.91 | 243 | 14.1 | 21.9 | 1.1 | 10.6 | 5.1 | 1.38 |
| Cheesy Bites (14") | 1107 | 8 | 336 | 19.6 | 30.2 | 1.6 | 14.7 | 7.0 | 1.91 | 243 | 14.1 | 21.9 | 1.1 | 10.6 | 5.1 | 1.38 |
| Gluten Free (9" Square) | 474 | 6 | 196 | 7.5 | 21.2 | 1.4 | 8.4 | 3.1 | 1.05 | 249 | 9.5 | 26.8 | 1.8 | 10.6 | 3.9 | 1.33 |

# SUBWAY® Nutrition Information

| LOW FAT SUBS per 6-inch | Energy (kJ) | Energy (kcal) | Fat (g) | of which Saturates (g) | Sugars (g) | Salt (g) |
|---|---|---|---|---|---|---|
| Chicken Breast | 1279 | 306 | 3.7 | 1.5 | 7.1 | 1.2 |
| Chicken Teriyaki | 1347 | 322 | 3.8 | 1.5 | 10.0 | 1.7 |
| Chicken Tikka | 1287 | 310 | 3.7 | 1.3 | 7.8 | 1.3 |
| Ham‡ | 1210 | 290 | 4.4 | 1.0 | 7.5 | 1.6 |
| Turkey Breast‡ | 1158 | 277 | 3.2 | 1.2 | 7.8 | 1.4 |
| Turkey Breast‡ & Ham‡ | 1226 | 293 | 3.9 | 1.4 | 7.3 | 1.6 |
| VEGGIE DELITE® | 934 | 221 | 3.2 | 1.0 | 6.7 | 0.6 |

| SUBS per 6-inch | Energy (kJ) | Energy (kcal) | Fat (g) | of which Saturates (g) | Sugars (g) | Salt (g) |
|---|---|---|---|---|---|---|
| Big Beef Melt‡ | 1666 | 403 | 15.3 | 8.1 | 7.9 | 1.6 |
| Chicken Bacon Ranch Melt | 2100 | 503 | 19.7 | 8.3 | 7.3 | 2.4 |
| Chicken Pizziola (includes American Style Processed Cheese) | 1853 | 443 | 14.4 | 6.7 | 9.8 | 2.3 |
| Italian B.M.T.® | 1725 | 412 | 17.7 | 7.3 | 7.3 | 2.4 |
| Meatball Marinara | 1838 | 439 | 16.2 | 6.8 | 13.6 | 1.9 |
| Spicy Italian | 2019 | 482 | 26.2 | 11.0 | 7.0 | 2.7 |
| Steak‡@ & Cheese (includes American Style Processed Cheese) | 1483 | 355 | 7.0 | 3.7 | 9.0 | 1.7 |
| SUBWAY MELT™ (includes American Style Processed Cheese) | 1562 | 373 | 10.2 | 4.7 | 7.8 | 2.2 |
| Tuna (with Lite Mayonnaise) | 1469 | 354 | 11.8 | 1.8 | 7.3 | 1.8 |
| Veggie Patty* | 1592 | 381 | 10.7 | 3.0 | 8.0 | 1.8 |

| KIDS' PAK™ MINI SUBS | Energy (kJ) | Energy (kcal) | Fat (g) | of which Saturates (g) | Sugars (g) | Salt (g) |
|---|---|---|---|---|---|---|
| Ham‡ | 700 | 162 | 2.6 | 1.0 | 4.9 | 0.9 |
| Turkey Breast‡ | 772 | 184 | 2.1 | 0.6 | 4.7 | 0.9 |
| VEGGIE DELITE® | 618 | 147 | 1.5 | 0.7 | 4.5 | 0.4 |

| LOW FAT SALADS per portion | Energy (kJ) | Energy (kcal) | Fat (g) | of which Saturates (g) | Sugars (g) |
|---|---|---|---|---|---|
| Chicken Breast | 573 | 137 | 2.3 | 0.6 | 6.4 |
| Chicken Tikka | 591 | 141 | 2.3 | 0.5 | 6.9 |
| Ham‡ | 507 | 121 | 3.1 | 0.8 | 6.9 |
| Chicken Teriyaki | 640 | 153 | 2.4 | 0.7 | 9.4 |
| Turkey Breast‡ | 452 | 108 | 1.8 | 0.4 | 6.4 |
| Turkey Breast‡ & Ham‡ | 518 | 124 | 2.0 | 0.6 | 6.7 |
| VEGGIE DELITE® | 217 | 52 | 0.9 | 0.2 | 6.1 |

| SALADS per portion | Energy (kJ) | Energy (kcal) | Fat (g) | of which Saturates (g) | Sugars (g) |
|---|---|---|---|---|---|
| Big Beef Melt‡ | 979 | 234 | 14.0 | 7.3 | 7.2 |
| Chicken & Bacon Ranch Melt | 1397 | 334 | 19.4 | 7.5 | 6.8 |
| Chicken Pizziola (includes American Style Processed Cheese) | 1147 | 274 | 13.1 | 5.8 | 9.2 |
| Italian B.M.T.® | 1019 | 244 | 16.4 | 6.5 | 6.7 |
| Meatball Marinara | 1131 | 270 | 14.8 | 6.0 | 12.9 |
| Spicy Italian | 1312 | 314 | 24.9 | 10.2 | 6.4 |
| Steak‡@ & Cheese (includes American Style Processed Cheese) | 777 | 186 | 6.7 | 2.9 | 8.4 |
| SUBWAY MELT™ (includes American Style Processed Cheese) | 855 | 205 | 8.9 | 3.9 | 7.2 |
| Tuna (with Lite Mayonnaise) | 783 | 187 | 10.3 | 1.0 | 6.7 |
| Veggie Patty* | 886 | 212 | 9.4 | 2.2 | 7.4 |

| BREADS per 6-inch | Energy (kJ) | Energy (kcal) | Fat (g) | of which Saturates (g) | Sugars (g) |
|---|---|---|---|---|---|
| 6-inch Italian (White) Bread | 820 | 196 | 1.9 | 0.9 | 4.0 |
| 6-inch 9-Grain Wheat Bread | 858 | 205 | 2.1 | 1.0 | 4.7 |
| 6-inch 9-Grain Honey Oat | 914 | 218 | 2.3 | 1.0 | 6.3 |
| 6-inch Hearty Italian Bread | 876 | 209 | 2.0 | 0.9 | 5.0 |
| 6-inch Italian Herb & Cheese | 1014 | 242 | 4.9 | 2.7 | 5.1 |
| Flatbread | 932 | 220 | 2.5 | 0.3 | 4.4 |

## Yo! Sushi

### ROLLS. Large & small nori rolls with rice

| Description | Energy | Each portion contains Fat | Saturates | Sugars | Salt | Cereals Containing Gluten | Crustaceans | Eggs | Fish | Peanuts | Soya | Milk | Nuts | Celery | Mustard | Sesame Seeds | Sulphur Dioxide | Lupin | Molluscs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Crispy Salmon Skin Roll | 114kcal | 2.5g | 0.4g | 2.7g | 0.55g | | | | ✔ | | | | | | | ✔ | | | |
| California Roll | 142kcal | 5.9g | 0.4g | 2.6g | 0.88g | ✔ | | ✔ | ✔ | | ✔ | | | | ✔ | ✔ | | | |
| | | | | | | Contains: Wheat | | | | | | | | | | | | | |
| Spicy Chicken Roll | 130kcal | 3.2g | 0.4g | 2.6g | 0.87g | ✔ | | ✔ | | | | | | | | ✔ | | | |
| | | | | | | Contains: Wheat | | | | | | | | | | | | | |
| Seared Beef Roll | 161kcal | 1.7g | 0.5g | 5.6g | 1.2g | ✔ | | | | | ✔ | | | | | ✔ | | | |
| | | | | | | Contains: Wheat | | | | | | | | | | | | | |
| Smoked Salmon & Cream Cheese Roll | 220kcal | 13g | 7.6g | 2.7g | 1.8g | | | | ✔ | | | ✔ | | | | | | | |
| YO! Roll | 140kcal | 4.7g | 0.7g | 2.7g | 0.66g | ✔ | | ✔ | ✔ | | ✔ | | | | ✔ | | | | |
| | | | | | | Contains: Wheat | | | | | | | | | | | | | |
| Yasai Roll | 170kcal | 5.6g | 0.3g | 5.5g | 1.0g | ✔ | | ✔ | | | ✔ | | | | ✔ | | | | |
| | | | | | | Contains: Wheat | | | | | | | | | | | | | |
| Spicy Tuna Roll | 132kcal | 3.2g | 0.3g | 2.9g | 0.71g | | | ✔ | ✔ | | ✔ | | | | ✔ | ✔ | | | |
| Ebi Roll | 127kcal | 3.2g | 0.3g | 2.7g | 1.0g | ✔ | ✔ | ✔ | | | ✔ | | | | ✔ | | | | |
| | | | | | | Contains: Wheat | | | | | | | | | | | | | |

Zizzi

## • CLASSIC PIZZA •

Per serving if not stated otherwise in the menu dish name

| MENU ITEM | Energy | | Total Fat (g) | Saturated Fat (g) | Total Sugars (g) | Salt (g) |
|---|---|---|---|---|---|---|
| | Kcal | KJ | | | | |
| MARGHERITA CLASSIC | 852 | 3547 | 39 | 13 | 12 | 3.4 |
| PEPPERONI CAMPAGNA CLASSIC | 870 | 3637 | 29 | 12 | 10 | 4.1 |
| WINTER ZUCCA CLASSIC | 1173 | 4936 | 47 | 20 | 22 | 4.3 |
| ITALIAN HOT CLASSIC | 925 | 3858 | 36 | 18 | 9.4 | 4.2 |
| POLPETTE CLASSIC | 1224 | 5155 | 57 | 25 | 10 | 4.7 |

## • SKINNY PIZZA •

Per serving if not stated otherwise in the menu dish name

| MENU ITEM | Energy | | Total Fat (g) | Saturated Fat (g) | Total Sugars (g) | Salt (g) |
|---|---|---|---|---|---|---|
| | Kcal | KJ | | | | |
| SKINNY KING PRAWN DIAVOLA | 486 | 2053 | 13 | 6.3 | 5.6 | 3.5 |
| SKINNY POLLO ROSSO | 531 | 2229 | 16 | 7.2 | 6.3 | 2.7 |
| SKINNY PRIMAVERA | 521 | 2181 | 18 | 4.8 | 14 | 2.5 |

## • SALADS •

Per serving if not stated otherwise in the menu dish name

| MENU ITEM | Energy | | Total Fat (g) | Saturated Fat (g) | Total Sugars (g) | Salt (g) |
|---|---|---|---|---|---|---|
| | Kcal | KJ | | | | |
| SUPER ZUCCA SALAD | 572 | 2405 | 21 | 8.4 | 14 | 1.9 |
| CHICKEN & PROSCIUTTO SALAD | 397 | 1660 | 23 | 3.9 | 2.8 | 2.4 |
| GREEN GODDESS SALAD (BROCCOLI) | 256 | 1064 | 16 | 1.9 | 6.0 | 0.7 |
| GREEN GODDESS SALAD (SALMON) | 594 | 2491 | 35 | 5.4 | 3.2 | 1.4 |

*Unfortunately we do not hold the nutritional data for the Super Zucca Salad with chicken.

## • RUSTICA PIZZA •

Per serving if not stated otherwise in the menu dish name

| MENU ITEM | Energy | | Total Fat (g) | Saturated Fat (g) | Total Sugars (g) | Salt (g) |
|---|---|---|---|---|---|---|
| | Kcal | KJ | | | | |
| PICCANTE RUSTICA | 1328 | 5561 | 58 | 25 | 13 | 6.5 |
| GARNOSO RUSTICA | 1319 | 5560 | 65 | 26 | 6.3 | 6.0 |
| PULLED PORK ROMA RUSTICA | 1543 | 6484 | 86 | 37 | 35 | 6.6 |
| PRIMAVERA RUSTICA | 1309 | 5641 | 68 | 25 | 13 | 5.4 |
| SOFIA RUSTICA | 1320 | 5575 | 51 | 23 | 7.7 | 7.1 |
| MARGHERITA RUSTICA | 883 | 3690 | 29 | 15 | 10 | 3.5 |
| PEPPERONI CAMPAGNA RUSTICA | 1039 | 4344 | 39 | 17 | 12 | 5.5 |
| WINTER ZUCCA RUSTICA | 1494 | 6298 | 69 | 28 | 10 | 4.7 |
| ITALIAN HOT RUSTICA | 1138 | 4757 | 54 | 26 | 9.1 | 5.0 |
| POLLO ROSSO RUSTICA | 1405 | 5914 | 62 | 24 | 13 | 5.9 |
| POLPETTE RUSTICA | 1514 | 6373 | 73 | 35 | 10 | 10.1 |

## • SIDES •

Per serving if not stated otherwise in the menu dish name

| MENU ITEM | Energy | | Total Fat (g) | Saturated Fat (g) | Total Sugars (g) | Salt (g) |
|---|---|---|---|---|---|---|
| | Kcal | KJ | | | | |
| TENDERSTEM BROCCOLI | 36 | 152 | 2.0 | 0.3 | 0.1 | 0.18 |
| MIXED LEAF, TOMATO & SPRING ONION SALAD | 64 | 268 | 5.5 | 0.8 | 1.8 | 0.36 |
| ROCKET & GRANA PADANO SALAD | 133 | 558 | 11 | 3.3 | 0.1 | 0.26 |
| ITALIAN NAKED SLAW | 22 | 93 | 0.2 | 0.1 | 3.8 | 0.39 |
| TUSCAN POTATOES | 225 | 941 | 6.5 | 2.1 | 2.9 | 0.24 |
| GREEN BEANS | 15 | 61 | 0.1 | 0 | 3.3 | 0.02 |
| BUFALA MOZZARELLA, TOMATO & BASIL SALAD | 248 | 1038 | 22 | 9.6 | 3.2 | 0.60 |
| BUTTERED MASH | 279 | 1168 | 18 | 12 | 3.7 | 1.3 |

CALIBER Menu 1– Low-carb, high-fat – Week 2

| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|
| BF | Scrambled eggs and parmesan cheese<br>Coffee (no sugar) with a little full-fat milk | Greek yoghurt, mixed berries and chia seeds<br>Coffee (no sugar) with a little full-fat milk | Judy's fabulous oatmeal<br>Coffee (no sugar) with a little full-fat milk | Greek yoghurt, mixed nuts and chia seeds<br>Coffee (no sugar) with a little full-fat milk | Judy's fabulous oatmeal<br>Coffee (no sugar) with a little full-fat milk | Breakfast tapas<br>Coffee (no sugar) with a little full-fat milk | Mushroom omelette<br>Coffee (no sugar) with a little full-fat milk |
| L | Turkey breast with mixed leaves salad and tomatoes | Mixed salad with left-over lamb | Charred Halloumi and pepper salad | Left-over spicy turkey meatballs<br>½ raw red pepper | Left-over ham, cheddar and spinach frittata with mixed salad | Spring green and spinach tagine | Roast beef with chipotle butter and mixed vegetables |
| D | Chicken and broccoli risotto | Mushroom, pak choi and mangetout stir-fry | Spicy turkey meatballs in tomato sauce | Ham, cheddar and spinach frittata with mixed salad | Sausage stroganoff with cauli-mash and steamed carrots | Bacon-wrapped chicken with boursain filling, green beans and walnuts, sautéed tomatoes with herbs | Shakshuka |
| Snack | Devilled nuts,<br>1 bag of Pork scratchings,<br>2 handfuls of strawberries | 2 x mini baby bel<br>1 handful of walnuts<br>1 medium banana | Vegetable sticks (red peppers, carrot sticks, cucumber) with ranch dip<br>1 handful brazil nuts | Salami, cheese and cucumber rolls<br>1 handful of almonds<br>2 handful of raspberries | Avocado,<br>1 handful of olives<br>1 small apple | 1/2 tub of olives with cheese, nutty crackers with Tex Mex dip | 2x mini baby bel, vegetable sticks, 1 handful of walnuts |
| Drink | Bullet-proof coffee, Coffee (no sugar) with a little full-fat milk or cream; Tea (no sugar) with a little full-fat milk or unsweetened herbal teas; Water (unflavoured or flavoured with citrus fruit, mint) | | | | | | |

CALIBER Menu 1– Dietary guidelines

| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|
| BF | Porridge with semi-skimmed milk<br>1 slice of wholemeal toast with polyunsaturated reduced fat spread and jam<br>Tea with semi-skimmed milk | Overnight oats with mixed berries and chia seeds<br>Tea with semi-skimmed milk | Porridge with semi-skimmed milk<br>1 slice of wholemeal toast with polyunsaturated reduced fat spread and honey<br>Tea with semi-skimmed milk | Porridge with semi-skimmed milk<br>1 slice of wholemeal toast with polyunsaturated reduced fat spread and jam<br>Tea with semi-skimmed milk | Overnight oats with raspberries and almonds<br>Coffee with skimmed milk | Porridge with semi-skimmed milk<br>1 slice of wholemeal toast with polyunsaturated reduced fat spread and honey<br>Tea with semi-skimmed milk | Cornflakes with semi-skimmed milk<br>1 slice of wholemeal toast with polyunsaturated reduced fat spread and jam<br>Orange juice<br>Tea with semi-skimmed milk |
| L | Broccoli, pea and basil soup with 2 slices of wholemeal bread with polyunsaturated reduced fat spread | Fennel, cucumber and dill salad with roasted chickpeas and a wholemeal roll | White bean salad with garlic and kale with ciabatta bread | Left-over quinoa and salmon cakes on wholemeal roll with lettuce, tomato, cucumber and crème fraiche | Polenta with tomato, mozzarella, parma ham and pesto | Courgette, feta and oregano frittata with mixed salad | Chicken breasts stuffed with spinach and goats cheese with potatoes, carrots and courgette and garlic gratin |
| D | Mushroom and barley risotto with parmesan, Ciabatta bread and rocket and cherry tomato salad | Left-over Broccoli, pea and basil soup with 2 slices of wholemeal bread with polyunsaturated reduced fat spread | Quinoa and salmon cakes with sweet baked potato, low-fat crème fraiche and rocket | Roast lamb and summer vegetables | Italian hake bake with sautéed cherry tomatoes and herbs, steamed broccoli and brown rice | Lemon-Parsley bean salad with pitta bread | Left-over Courgette, feta and oregano frittata with mixed salad |
| Snack | 2 slices of wholemeal toast with marmite<br>1 banana, 1 apple, 2 biscuits | 1 pears, Grapes, 1 small handful of walnuts, 1 small pot of low-fat Greek yoghurt<br>2 biscuits | 1 banana, Strawberries,<br>1 Orange<br>1 small handful of cashew nuts<br>Crackers with hummus and veg sticks<br>2 biscuits | 2 apricots, 1 banana, 1 small slice of reduced fat cheese, Roasted chickpeas, 2 biscuits | Strawberries, 2 apricots, 1 small handful of Brazil nuts, 1 apple<br>2 biscuits | 1 Kiwi, 1 peach, 1 apple, 2 biscuits<br>1 small pot of low-fat Greek yoghurt with 1 tablespoon of chia seeds | 2 apricots<br>1 nectarine<br>Strawberries<br>1 small handful of Brazil nuts |
| Drink | Coffee (no sugar) with semi-skimmed milk; Tea (no sugar) with semi-skimmed milk; Water (unflavoured or flavoured with citrus fruit, mint) | | | | | | |

Composition of daily multivitamin and mineral supplement* given to participants in the LCHF group

| Ingredients | per tablet** | % NRV[*] |
|---|---|---|
| Vitamin A (RE) (50% as beta-carotene) | 800 µg | 100% |
| Vitamin E (α-TE) | 16 mg | 133% |
| Vitamin C | 80 mg | 100% |
| Vitamin K | 30 µg | 40% |
| Thiamine | 1.5 mg | 136% |
| Riboflavin | 1.8 mg | 129% |
| Vitamin B6 | 2.1 mg | 150% |
| Vitamin B12 | 7.5 µg | 300% |
| Vitamin D | 15 µg | 300% |
| Biotin | 75 µg | 150% |
| Folic acid | 300 µg | 150% |
| Niacin (NE) | 20 mg | 125% |
| Pantothenic acid | 9 mg | 150% |
| Calcium | 340 mg | 43% |
| Phosphorus | 105 mg | 15% |
| Magnesium | 107 mg | 29% |
| Iron | 4.2 mg | 30% |
| Iodine | 100 µg | 67% |
| Copper | 500 µg | 50% |
| Manganese | 2.4 mg | 120% |
| Chromium | 40 µg | 100% |
| Molybdenum | 50 µg | 100% |
| Selenium | 30 µg | 55% |
| Zinc | 5 mg | 50% |

*Centrum Women 50+

**Participants were advised to take one tablet per day, preferably at meal time.

[*] European RDA

## Appendix B: Conference publications

*Proceedings of the Nutrition Society* (2016), 75 (OCE3), E237 doi:10.1017/S0029665116002524

*Summer Meeting, 11–14 July 2016, New technology in nutrition research and practice*

# Nutritional status, dietary intake and adiposity of normal-weight individuals with clustered metabolic risk factors in the UK population

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In recent years, a number of novel adiposity proxies, such as the 'A Body Shape Index' (ABSI), Body Roundness Index (BRI) and Waist-hip-height ratio (WHHR) have been applied as an alternative to Body Mass Index (BMI), which has been challenged as proxy indicator of CM risk[1,2]. The 'thin outside, fat inside' (TOFI) obesity phenotype is characterised by BMI 18·5–24·9 kg/m$^2$ (normal weight), whilst manifesting a cluster of health-detrimental CM abnormalities[3,4]. The aim of this study was to investigate associations between dietary intake, nutritional status and CM risk in the normal weight UK population with and without clustered CM risk factors and to verify the suitability of novel adiposity proxies for the identification of TOFIs.

Using the National Diet and Nutrition Survey (NDNS) Rolling Programme 2008/09-2011/12 (unweighted data) a subsample (n 227) of the normal weight population was analysed. Variation in nutritional status was investigated by the existence of the cluster CM risk[4]. Beside energy and nutrient intake, emerging (carotenoids)[5] and more established (e.g. plasma Vitamin D, ferritin and dietary carbohydrates[6]) correlates of CM risk were investigated. Traditional (waist circumference (WC)) and novel (ABSI, BRI and WHHR) measures of adiposity were also compared amongst groups using an independent t-test.

| | Males (n 78) | | | | Females (n 149) | | | |
|---|---|---|---|---|---|---|---|---|
| | TOFI* (n 43) | | Non-TOFI (n 35) | | TOFI* (n 49) | | Non-TOFI (n 100) | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| **Age (y)** | 45·0$^a$ | 16·7 | 35·2$^a$ | 12·8 | 46·6 | 13·3 | 41·8 | 11·6 |
| **Weight (kg)** | 73·0$^a$ | 6·39 | 69·2$^a$ | 7·30 | 60·2 | 5·9 | 60·0 | 6·3 |
| **Nutritional status** | | | | | | | | |
| Plasma Ferritin (umol/L) | 111·03 | 69·5 | 111·18 | 67·8 | 75·0$^b$ | 64·3 | 45·4$^b$ | 30·7 |
| 25-Hydroxy Vitamin D (nmol/L) | 40·2$^a$ | 25·1 | 53·6$^a$ | 25·6 | 51·4 | 30·4 | 54·1 | 26·8 |
| Total carotenoids (umol/L) | 2·3 | 0·9 | 2·3 | 1·2 | 2·1$^b$ | 0·9 | 2·9$^b$ | 1·3 |
| **Dietary Intake** | | | | | | | | |
| Total energy (kcal/d) | 2303·9 | 647·9 | 2169·7 | 490·2 | 1673·8 | 391·4 | 1726·2 | 405·3 |
| Food energy (kcal/d) | 2061·1 | 456·2 | 2099·2 | 446·7 | 1628·8 | 373·3 | 1650·5 | 391·1 |
| Total carbohydrates (%tot. energy) | 44·2$^a$ | 8·1 | 47·7$^a$ | 7·5 | 47·2 | 7·5 | 47·4 | 5·8 |
| Total sugars (% total energy) | 20·2 | 7·3 | 21·1 | 6·7 | 21·4 | 7·9 | 20·2 | 5·2 |
| NMES (% total energy) | 13·7 | 6·2 | 13·3 | 6·1 | 11·9 | 7·2 | 10·8 | 4·8 |
| **Adiposity measures** | | | | | | | | |
| ABSI ($m^{-2/3}$ $kg^{-2/3}$) | 0·080 | 0·005 | 0·078 | 0·006 | 0·077 | 0·005 | 0·076 | 0·006 |
| BRI | 3·4$^b$ | 0·7 | 2·9$^b$ | 0·5 | 3·1$^a$ | 0·8 | 2·9$^a$ | 0·6 |
| WC (cm) | 87·6$^b$ | 6·4 | 82·8$^b$ | 4·9 | 78·4 | 6·3 | 77·0 | 6·0 |
| WHHR ($m^{-1}$) | 0·51$^a$ | 0·04 | 0·48$^a$ | 0·03 | 0·50$^a$ | 0·04 | 0·48$^a$ | 0·04 |

$^a$ $p<0.05$ $^b$ $p<0.01$; * TOFI ≥2 CM abnormalities (Triglycerides >1·7 mmol/L; HDL <1·0 mmol/L (M)/<1·3 mmol/L (F) or lipid lowering medication; Systolic/diastolic blood pressure ≥130/85 mm Hg or antihypertensive medication; CRP >1 mg/L; Glucose ≥5·6 mmol/L)[4].

Female TOFIs had significantly higher levels of plasma ferritin but significantly lower levels of total carotenoids than non-TOFIs. For male TOFIs, plasma Vitamin D was significantly lower than for non-TOFIs. Carbohydrate intake (%total energy) was significantly lower in male TOFIs than non-TOFIs. For men only, TOFIs were significantly older and heavier and their BRI, WC and WHHR were significantly higher. For women, only BRI and WHHR were significantly higher in TOFIs. ABSI did not prove to be a useful proxy for adiposity for this group.

Diet quality and composition in this obesity phenotype warrant further investigation to establish how they might impact upon adipose tissue quality, distribution and associated risk factors for CM health. To improve identification of normal-weight individuals at risk, gender specific novel proxy indicators of adiposity may be required.

1 Song X, Jousilahti P & Stehouwer CD *et al.* (2013) *Eur J Clin Nutr.* 67, 1298–1302
2 Thomas DM, Bredlau C & Bosy-Westphal A *et al.* (2013) *Obesity (Silver Spring)* 21, 2264–2271
3 Thomas EL, Frost G & Taylor-Robinson SD *et al.* (2012) *Nutr Rev Rev.* 25, 150–61
4 Wildman RP, Muntner P & Reynolds K *et al.* (2008) *Arch Intern Med.* 168, 1617–1624
5 Giammona MA, Riccioni G & D'Orazio N (2015) *Food Nutr Res* 59, 26762
6 Mozaffarian D (2016) *Circulation.* 133, 187–225

*Proceedings of the Nutrition Society* (2017), 76 (OCE4), E198 doi:10.1017/S0029665117003603

*Summer Meeting, 10–12 July 2017, Improving Nutrition in Metropolitan Areas*

# Macronutrient intake and prevalence of markers of metabolic syndrome in white UK adult males in the National Diet and Nutrition Survey Rolling Programme 2008–2014

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The amount of carbohydrates recommended for consumption by current dietary guidelines has been challenged in relation to their suitability to prevent or manage cardiometabolic (CM) diseases with suggestions that they should be decreased and replaced by protein or fat[1,2]. Others have argued that a more personalised approach is required[3]. Aim of this investigation was to assess the potential impact of lower versus higher consumption of dietary macronutrients and prevalence of CM risk markers in a representative sample of the UK male white population.

Unweighted data from 642 white adult males aged 19 and over in the National Diet and Nutrition Survey Rolling Programme[4,5] (NDNS RP) 2008–2014 with or without metabolic syndrome (MetS)[6] were analysed for associations of dietary macronutrient intake as percentage food energy (%FE) with CM risk markers. Logistic regression analysis (adjusted for age group and smoking status) was used to compare the odds ratios [OR] of prevalence of individual markers of MetS between the lowest and highest quartiles of dietary macronutrient intake as %FE (≤44 vs. ≥52 for carbohydrates; ≤31 vs. ≥39 for fats; ≤15. vs. ≥19 for protein).

There was a significant ($p < 0.05$) reduction in likelihood of MetS (OR, .55; 95 % confidence interval [CI], .34 to .84), and elevated waist circumference (OR, .50; 95 % CI, .30 to .83) and glucose levels (OR, .51; 95 % CI, .30 to .87) for those in the highest quartile of carbohydrate %FE intake compared to the lowest quartile, whereas those in the highest quartile of protein %FE intake had a significantly ($p < 0.05$) increased risk of presenting with the same markers of MetS (OR, 1·75; 95 % CI, 1·05 to 2·93; OR, 2·12; 95 % CI, 1·24 to 3·63; and OR, 2·15; 95 % CI, 1·25 to 3·70 respectively). Those with the highest compared to the lowest total dietary fat intake also presented with elevated CM risk markes, albeit these findings were not significant.

| | CHONFE | | | FATNFE | | | PROTNFE | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 95 % CI for OR | | | 95 % CI for OR | | | 95 % CI for OR | |
| | OR | Lower | Upper | OR | Lower | Upper | OR | Lower | Upper |
| MetS* | .55* | .34 | .84 | 1·58 | .97 | 2·55 | 1·75* | 1·05 | 2·93 |
| TRIG | .72 | .46 | 1·14 | 1·36 | .86 | 2·15 | 1·18 | .73 | 1·91 |
| HDL-C | 1·13 | .69 | 1·87 | 1·19 | .71 | 1·98 | .86 | .50 | 1·47 |
| WC | .50* | .30 | .83 | 1·28 | .78 | 2·10 | 2·12* | 1·26 | 3·63 |
| GLUC | .51* | .30 | .87 | 1·46 | .86 | 2·47 | 2·15* | 1·25 | 3·70 |
| BP | .89 | .53 | 1·49 | 1·38 | .81 | 2·36 | 1·19 | .68 | 2·06 |

*Metabolic Syndrome (MetS) definition: 3 out of 5 of the following: triglycerides (TRIG) ≥1·7 mmol/l; High-density lipoprotein cholesterol (HDL-C) ≤1·03 mmol/l. for males; Waist circumference (WC) ≥94 cm for white males; Glucose (GLUC) ≥5·6 mmol/l.; Blood pressure (BP) ≥130 mmHg systolic or ≥85 mmHg diastolic respectively; CHONFE – total carbohydrates percentage food energy; FATNFE – total fats food energy; PROTNFE– total protein food energy; OR – odds ratio (adjusted for age group and smoking status), 1st vs. 4th quartile of intake; CI – confidence interval; a $p < 0.05$

Further investigations need to confirm whether the quality of the macronutrients consumed and overall diet quality[6] has had an impact on these results. In the context of a personalised approach to nutrition future cohort studies should also provide data that allow for examining inter-individual variations in responses to dietary macronutrients, especially carbohydrates, to achieve optimum CM health for a larger proportion of the population.

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*Proceedings of the Nutrition Society* (2018), 77 (OCE4), E208 doi:10.1017/S0029665118002148

*Summer Meeting, 10–12 July 2018, Getting energy balance right*

# The association between dietary macronutrient intake and fibrogen growth factor 21 in a sample of White UK adults with elevated cardiometabolic risk markers

T. Harrison[1], D. McCullough[1], K.E. Lane[1], L.M. Boddy[2], C.E. Stewart[2], K.J. Enright[1], F. Amirabdollahian[3], M.A. Schmidt[4] and I.G. Davies[1]

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Increased levels of Fibrogen growth factor 21 (FGF21) is an emerging risk marker for cardiometabolic (CM) disease[1]. Little detail is known about the impact of the human diet on FGF21 levels. The aim of this investigation was to assess potential associations between mean daily dietary macronutrient intake and FGF21 levels in a sample of 10 healthy normal-weight and overweight Caucasian adults aged 32–60 (80 % male) at increased CM risk[2]. This pilot study received ethical approval from Liverpool John Moores University Research Ethics Committee (16/ELS/029) and was registered with ClinicalTrials.gov (Ref. NCT03257085).

Participants were randomly allocated to one of two groups and asked to either consume < 50 g/d of dietary CHO (low-carb (LC)) or to follow the UK dietary guidelines and obtain >50 % energy from CHO for a duration of 8 weeks. Blood plasma samples were collected at baseline (BL), interim point (IP) and endpoint (EP) after a 12-hour overnight fast, immediately processed and frozen at −80°C. Thawed plasma samples were analysed via Quantikine® enzyme-linked immunosorbent assay (ELISA) (R&D Systems) for FGF21 levels. Two-way mixed ANOVA and Pearson's partial correlation adjusted for estimated weekly moderate and vigorous activity was undertaken using IBM SPSS 24®.

There were no effects for diet between groups or over time (data not shown). Significant correlations between macronutrient intakes and FGF21 levels were found for both groups at IP, but not at BL or EP. Moderate and significant positive correlations were found in the overall group for intake (g/d) for glucose ($r_{partial}$ = 699, p = .04) and fructose ($r_{partial}$ = 686, p = .04) and strong and significant positive correlations for non-milk extrinsic sugars ($r_{partial}$ = .742, p = .02). Strong and significant positive correlations were also found in the LC group for glucose intake (g/d) ($r_{partial}$ = .980, p = 0.2) and fructose ($r_{partial}$ = 967, p = .03) and for protein ($r_{partial}$ = .998, p = .002) after adjusting for physical activity. Mean carbohydrate intake (g/d) was 160·0 (s.d. 124·5) overall and 44·2 (s.d. 14·9) in the LC group at IP. Mean protein intake (g/d) was 113·2 (21·4) 130·0 (s.d. 15·9) overall and in the LC group at IP. Mean FGF21 levels were 179·9 pg/mL (s.d. 144·9) in the overall group and 94.4 pg/ML (s.d. 48.6) in the LC group at IP.

| | Total kcal | %TE | | | | Intake (g/d) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CHO | NMES | PROT | FAT | CHO | GLU | FRU | NMES | PROT | FAT |
| | r | r | r | r | r | r | r | r | r | r | r |
| T | −.216 | .628 | .635 | −.326 | −.491 | .468 | .699* | .686* | .742* | −.006 | −.496 |
| LC | .146 | .637 | .937 | .427 | −.059 | .722 | .980* | .967* | .919 | .998** | −.080 |

CHO-Total carbohydrate, FAT-Total fat, FRU-Fructose, GLU-Glucose, LC-low-carbohydrate, high-fat group, NMES-non-milk extrinsic sugars, PROT-protein, T – total, %TE – percentage total energy, *p < .05 **p < .005

In conclusion, low-carbohydrate diets provide the opportunity to assess responses to even small amounts of CHO, which are likely to be replaced in part by proteins. Despite low overall intakes of fructose and glucose in the LC group, strong and positive correlations with FGF21 levels were observed. The lower levels of FGF21 in the LC compared to the overall group are in line with findings that FGF21 levels are elevated with high-carbohydrate, low-protein diets with dietary fats having only minor impact[3]. However, the majority of studies have still been undertaken using rodent models. The impact of dietary macronutrients on FGF21 levels as novel CMR marker in humans and the mechanism behind this relationship warrant further investigation.

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*Proceedings of the Nutrition Society* (2018), 77 (OCE4), E169 doi:10.1017/S0029665118001751

*Summer Meeting, 10–12 July 2018, Getting energy balance right*

# Dietary carbohydrate intake, visceral adipose tissue and associated markers of cardiometabolic risk

T. Harrison[1], D. McCullough[1], K.E. Lane[1], L.M. Boddy[2], C.E. Stewart[2], K.J. Enright[1], F. Amirabdollahian[3], M.A. Schmidt[4] and I.G. Davies[1]

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Risk of cardiometabolic (CM) disease is characterised by elevated visceral adipose tissue (VAT) and a number of associated biomarkers[1]. Some dietary carbohydrates (CHO) have been found to contribute to VAT accumulation[2]. Little is known about the impact of following a low-carbohydrate diet versus a high-carbohydrate diet on VAT, adiponectin (ADPN), leptin (LEPT) and leptin:adiponectin ratio (LAR). The aim of this investigation was to assess the impact of dietary carbohydrates (CHO) on VAT and emerging CM risk markers in a sample of 10 healthy normal-weight and overweight Caucasian adults aged 32–60 (80 % male) at increased CM risk[3]. This pilot study received ethical approval from Liverpool John Moores University Research Ethics Committee (16/ELS/029) and was registered with ClinicalTrials.gov (Ref. NCT03257085).

Participants were randomly allocated to one of two groups and asked to either consume < 50 g/d of dietary CHO (low-carb (LC)) or to follow the UK dietary guidelines and obtain >50 % energy from CHO (high-carb (HC)) for a duration of 8 weeks. VAT was analysed via bioelectrical impedance (SECA mBCA 515). Blood plasma samples were collected at baseline (BL), interim point (IP) and endpoint (EP) after a 12-hour overnight fast, immediately processed and frozen at -80°C. Thawed plasma samples were analysed via immunoassay technology (Randox Evidence Investigator™ Metabolic Syndrome Arrays I and II) for ADPN and LEPT levels. Statistical analysis was undertaken using IBM SPSS 24®.

Parametric data was analysed via two-way mixed ANOVA; non-parametric data was analysed via Mann-Whitney U test and Friedman test. Average daily carbohydrate intake in the LC group was 44·2 g at IP and 48·9 g at EP.

There were no significant differences between groups at any time point for ADPN, LEPT, LAR or VAT and no significant interactions for time or group*time for ADPN, LEPT or LAR. However, in the LC group VAT decreased significantly between baseline and endpoint by 15 % ($p = 0·15$) Over the course of the intervention ADPN and LEPT decreased non- significantly (by 4 % and 70 % respectively) in the LC group, whilst increasing non-significantly in the HC group (9 % and 65 % respectively). LAR increased in the HC group throughout the study, whilst LAR in the LC group decreased albeit not significantly.

| | VAT (litre) | | | | | | ADPN (ug/ml) | | | LEPT (ug/ml) | | | LAR | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BL | | IP | | EP | | Median | | | Median | | | Median | | |
| | M | SD | M | SD | M | SD | BL | IP | EP | BL | IP | EP | BL | IP | EP |
| LC | 4·1* | 1·2 | 3·8 | 1·3 | 3·9 | 1·2 | 8·9 | 8·6 | 8·5 | 5·96 | 1·64 | 1·20 | 0·45 | 0·19 | 0·14 |
| HC | 2·7 | 0·1 | 1·6 | 0·3 | 2·5 | 0·1 | 11·3 | 13·4 | 12·3 | 0·97 | 1·1 | 1·40 | 0·07 | 0·07 | 0·46 |

ADPN = adiponectin, BL = baseline, EP = endpoint, HC = high-carbohydrate, moderate fat diet, IP = interim point, LAR = leptin:adiponectin ratio, LEPT = leptin, LC = low-carbohydrate, high-fat diet, VAT = visceral adipose tissue, *$p = 0·15$. NR: interquartile range not provided for median values due to missing data.

Higher LAR has been found to be a marker of increased CM risk[4]. In conclusion, while the significant reduction in VAT in the LC group corresponds with the reduction of LAR further evidence is required to corroborate these findings. Previous evidence for LC is supportive for improved CM health from various biomarkers[5]; LAR should be considered as a useful endocrine addition for future LC studies.

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*Proceedings of the Nutrition Society* (2019), **78 (OCE1)**, E16 doi:10.1017/S002966511900020X

*Winter Meeting, 4–5 December 2018, Optimal diet and lifestyle strategies for the management of cardio-metabolic risk*

# The effect of dietary carbohydrate manipulation on low-density lipoprotein-cholesterol and its associated cardiometabolic risk

D. McCullough[1,2], T. Harrison[1], K.E. Lane[1], L.M. Boddy[2], C.E. Stewart[2], K.J. Enright[1], F. Amirabdollahian[3], M.A. Schmidt[4] and I.G. Davies[1]

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Cardiometabolic (CM) risk is typically increased with elevated low-density lipoprotein-cholesterol (LDL-C) and insulin resistance (IR)[1,2]. A low carbohydrate, high fat (LCHF) diet has been shown to increase LDL-C albeit improving other CM risk factors such as high-density lipoprotein-cholesterol and triglycerides[3]. There are several subclasses of LDL, in which some may be more atherogenic such as small-dense LDL (sdLDL)[4]. Few studies have compared a LCHF diet to a high carbohydrate, lower fat diet under *ad libitum* conditions, particularly their effect on sdLDL:LDL-C ratio. The current feasibility study intends to address such gap. Furthermore, to the authors' knowledge, the effect of the new reduced sugar UK Eatwell guide on CM health is yet to be investigated. Therefore, the aims of this investigation was to measure the effect of a low carbohydrate (LC) diet vs. a high carbohydrate (HC) diet on LDL-C, sdLDL-C and IR in 16 (9 males, 7 females) healthy Caucasian adults aged 19-64.

The study received ethical approval from Liverpool John Moores University Research Ethics Committee (16/ELS/029) and was registered with ClinicalTrials.gov (Ref. NCT03257085). Participants were randomly assigned to either a HC diet (the UK Eatwell guidelines; ≥50 % of energy from carbohydrates) (n = 8, 5 males, 3 females), or a LC diet (consume <50 g/day of carbohydrates) (n = 8, 4 males, 4 females) for 8 weeks. At 0, 4 and 8 weeks blood was collected after a 12 hour fast, processed for plasma and stored at -80ºC. Plasma was analysed by an automated chemistry analyser (Daytona, Randox Laboratories Ltd, UK) for LDL-C, sdLDL-C and glucose levels. Insulin levels were measured using immunoassay technology (Randox Evidence Investigator™ Metabolic Syndrome Arrays I). The homeostatic model assessment (HOMA) was used to calculate IR. Statistical analysis was undertaken using IBM SPSS 24®. Normally distributed data underwent a 2 × 3 mixed ANOVA to investigate significant differences for effect of time and interaction effect. Spearman's correlation was used to analyse the association between variables.

LDL-C non-significantly ($P = 0.141$) increased by 0.22 (mmol/L) within the LC group whereas the HC group remained unchanged. Within the LC group sdLDL-C levels decreased by 0.14 (mmol/L); however, sdLDL-C in the HC group increased by 0.07 (mmol/L) resulting in a significant interaction effect ($P = 0.026$). The ratio of sdLDL:LDL-C therefore decreased by 0.06 in the LC group and increased by 0.01 in the HC group resulting in a significant interaction effect ($P = 0.003$). HOMA significantly improved ($P = 0.008$) similarly in both groups but the change in HOMA was only significantly ($R^2 = 0.988$, $P = 0.008$) associated with the change in sdLDL: LDL-C within the LC group.

In conclusion, the study provided preliminary evidence showing that a LC diet may improve CM health via positive changes in LDL composition with an associated reduction in IR. Although the HC diet improved IR, the unfavourable changes in LDL size may indicate only a partial improvement in CM health. Further research is required on how dietary carbohydrate manipulation can improve LDL composition and overall CM health. The use of sdLDL:LDL-C ratio may be of more importance when assessing improvements in CM health compared to LDL-C alone.

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*Proceedings of the Nutrition Society* (2016), 75 (OCE3), E230 doi:10.1017/S0029665116002457

*Summer Meeting, 11–14 July 2016, New technology in nutrition research and practice*

# Traditional and novel correlates of adiposity and cardiometabolic risk among young healthy adults in the North West of England

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Excessive adiposity is associated with increased cardiometabolic (CM) risk[1]. The discriminatory power of traditional proxy indicators of adiposity such as Body Mass Index (BMI), Waist Circumference (WC) and Waist to Hip Ratio (WHpR) has been frequently challenged[2,3]. In recent years, several novel proxy measures of adiposity such as Waist to Height Ratio (WHtR)[2], Clinica Universidad de Navarra – Body Adiposity Estimator (CUN-BAE)[3] and A Body Shape Index (ABSI)[4] and have been suggested as alternatives to the traditional measures. The aim of this study was to investigate which proxy measure of anthropometric adiposity has the strongest association with CM risk indices in healthy young adults in North West England.

After obtaining ethical approval, 396 (171 male and 225 female) participants aged 18–24 years were recruited in a cross-sectional study. Anthropometric, dietary and laboratory measures of CM risk were assessed including: percentage body fat (%BF measured via bioelectrical impedance Tanita™), blood pressure (BP), 3-day validated food diary and fasting capillary whole blood glucose and lipid profile. Traditional (BMI, WC, WHpR) and novel (CUN-BAE, ABSI and WHtR) proxy indicators of adiposity were assessed or calculated using standardised techniques[2–4]. The strength of the association of these measures with CM risk indices were then compared based on the strength of the Pearson correlation coefficient in males (M) and females (F) (Table 1).

**Table 1.** Pearson correlation coefficient of the association between cardiometabolic risk indices and proxy indicators of adiposity (*p < 0.05, **p < 0.01).

| | BMI kg/m2 | | WC cm | | WHpR % | | CUNBAE ml kg-1 min-1 | | ABSI | | WHtR | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F | M | F | M | F |
| **Anthropometry** | | | | | | | | | | | | |
| %BF | .25** | .41** | .29** | .48** | 0.06 | 0 | .26** | -.38** | 0.15 | 0.01 | .31** | .59** |
| Systolic BP (mmHg) | 0.02 | 0.07 | 0 | .13* | −0.11 | 0.07 | 0.05 | 0.12 | −0.06 | 0.03 | −0.02 | 0.07 |
| Diastolic BP (mmHg) | 0.04 | .20** | 0.06 | .22* | 0.13 | .16** | 0.04 | .12* | 0.07 | 0.05 | 0.1 | .22** |
| **Dietary** | | | | | | | | | | | | |
| Average energy intake (kcal/d) | −0.05 | 0 | −0.1 | 0.04 | −0.01 | −0.06 | −0.03 | −0.09 | −0.04 | 0.13 | −0.08 | 0.05 |
| Energy from fat (%) | −0.09 | 0.11 | 0.06 | .18** | 0 | 0.03 | −0.1 | 0.12 | -.15* | 0.1 | 0.02 | .16** |
| Energy from saturated fat (%) | −0.11 | 0.1 | 0.02 | -.16* | −0.01 | 0.02 | −0.13 | 0.11 | 0.12 | 0.1 | −0.01 | -.15** |
| Energy from sugar (%) | −0.01 | −0.1 | −0.1 | −0.1 | 0.06 | −0.08 | −0.01 | −0.07 | −0.09 | −0.05 | −0.05 | −0.09 |
| **Laboratory** | | | | | | | | | | | | |
| Blood Cholesterol (mmol/L) | 0.15 | 0.05 | 0.11 | 0.1 | −0.01 | 0 | .16* | 0.1 | 0.02 | 0.06 | .16* | 0.09 |
| Blood TG (mmol/L) | 0.02 | 0.6 | 0.08 | 0.06 | 0.07 | −0.01 | 0.03 | 0.08 | 0.09 | −0.01 | 0.08 | 0.06 |
| Blood LDL (mmol/L) | 0.14 | 0.1 | 0.03 | 0.14 | −0.01 | −0.11 | 0.15 | .23** | −0.08 | −0.06 | 0.08 | 0.09 |
| Blood Glucose (mmol/L) | −0.04 | .15* | 0.11 | .19** | .16* | .32** | −0.05 | 0.12 | .17* | 0.12 | 0.09 | .19** |

For men, most novel and traditional proxy measures showed weak associations with measured %BF. While there were occasional correlations with other dietary and laboratory correlates of CM risk, both CUN-BAE and WHtR showed weak but significant association with %BF and whole blood total cholesterol. For women, CUN-BAE correlated the strongest with %BF, while WC and WHtR demonstrated weak but (very) significant associations with various anthropometric, dietary and laboratory indices of CM risk. The findings suggest that for young adults in general, ABSI and WHpR show no or limited potential as proxy indicators of adiposity. Furthermore, the findings propose that gender specific proxy indicators may be required and, specifically for women, use of WC, WHtR and CUN-BAE may be more appropriate than BMI. This might be due to differences in adipose tissue type and distribution.

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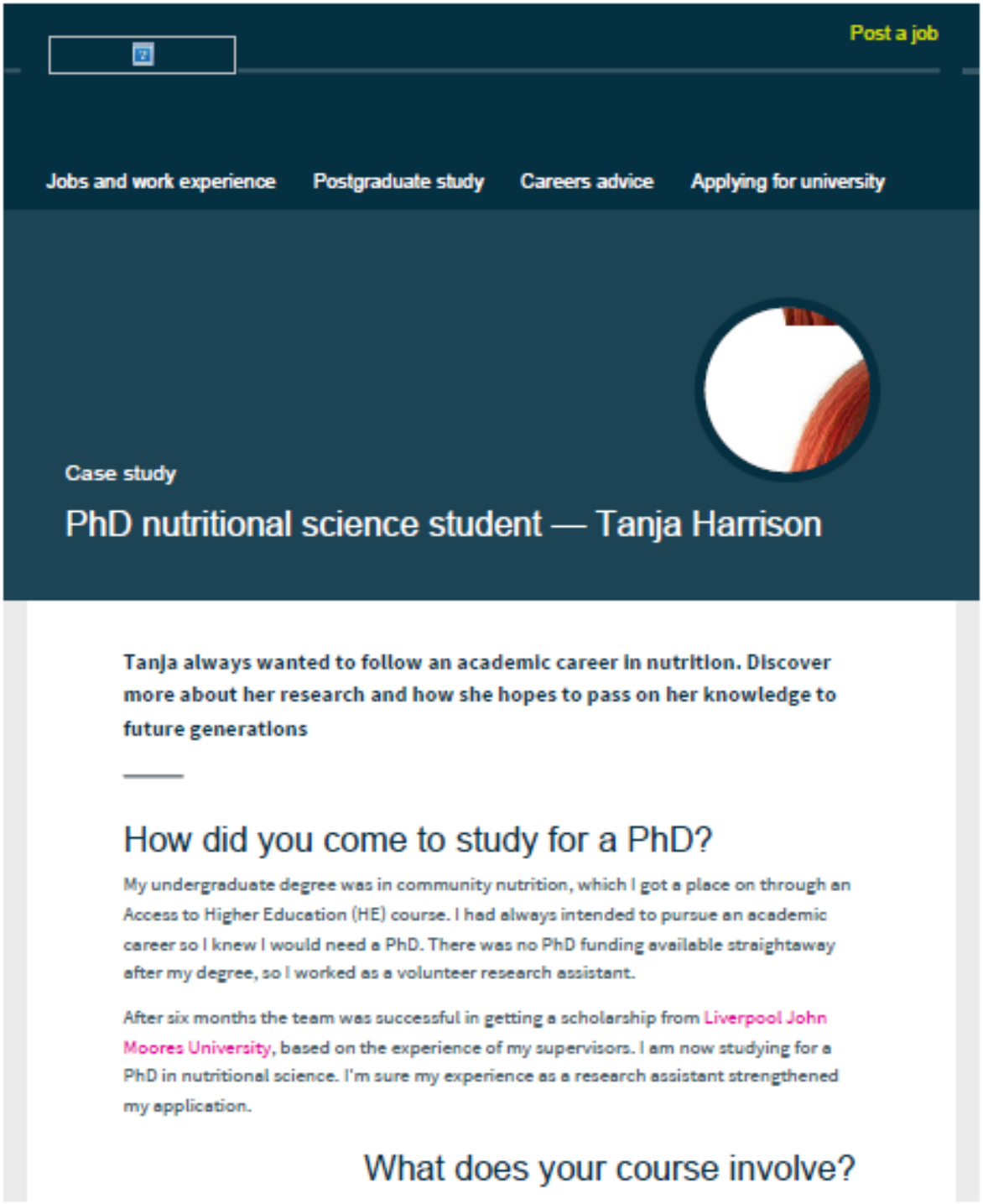
## Appendix C – Other communications

PhD nutritional science student: Tanja Harrison | Prospects.ac.uk




https://www.prospects.ac.uk/case-studies/phd-nutritional-science-student-tanja-harrison[10/04/2019 11:57:51]

"FUTURE DIETARY ADVICE MAY BE MORE PERSONALISED SO EVERYONE'S PLATE LOOKS DIFFERENT"

The focus of my research is on the way in which cardiometabolic risk markers, food cravings and carbohydrate intake are linked. Firstly, I analysed the National Diet and Nutrition Survey for the prevalence of normal-weight obese males. In stage 2, we are recruiting participants for a 16-week study where normal weight and overweight adult males will consume step-wise decreasing or increasing amounts of carbohydrates and fats to see which approach works best for the individual.

## Which parts of your degree have been most useful?

Learning how to take and analyse a food diary and provide evidence-based nutrition advice have proved invaluable for my PhD research, as was learning how to use different types of laboratory equipment.

## What do you enjoy most about your research?

I enjoy the public engagement. As always with research, it's not enough just to do the research; we also have to be able to communicate it effectively to our funders and, ultimately, get the message across to the public.

## What are the challenges?

It's always difficult when you try to challenge accepted dietary guidelines. For example, high carbohydrate/low fat may not be right for everyone. Future dietary advice may be more personalised so everyone's plate looks different.

## What are your future career plans?

I would like a university career, combining academic research and lecturing, to pass on the research and knowledge to the next generation of nutrition students.

My first step after my PhD is likely to be a postdoctoral job. I'll also be looking for advertised lecturing posts.

## Do you have any work experience?

While I was doing my degree, I volunteered at a food bank. It gave me a great insight into the real world of people's day-to-day lives. It was a good reality check reminding me that, for example, five-a-day guidelines are meaningless to those who are struggling to

feed a family.

Before my degree, I had considered becoming a dietitian so I shadowed at several different hospitals. I decided that hospital work was not for me, but the experience was fascinating.

## What's your advice for someone starting out?

- Join the Association for Nutrition (AfN) Register. Being registered led to a guest lectureship at a university, who approached me through the Register.
- Professional networking is essential. The nutrition world is a close-knit community. I've found that AfN events are useful. I co-organised and spoke at the AfN North West Group conference.
- Be proactive. Opportunities don't always present themselves. You have to go out and find them - or even create them. But when those opportunities arise, don't be afraid to take them. Say yes, and give it a go.

## Find out more

- Discover what you can do with a degree in nutrition.
- Learn more about PhD study.

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Sourced by Susanne Christian
AGCAS - April 2017


## You may also like…

# High-carb and low-carb diets and the risk of developing heart disease and Type 2 Diabetes

## Lifestyle-related diseases such as heart disease and Type 2 Diabetes are a big health problem in the UK

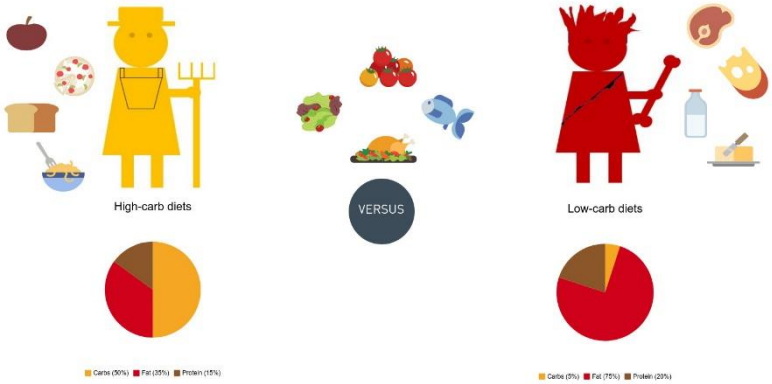
**42,245**
people with cardiovascular disease died prematurely in the UK in 2015

**7 million**
people in the UK currently live with cardiovascular disease

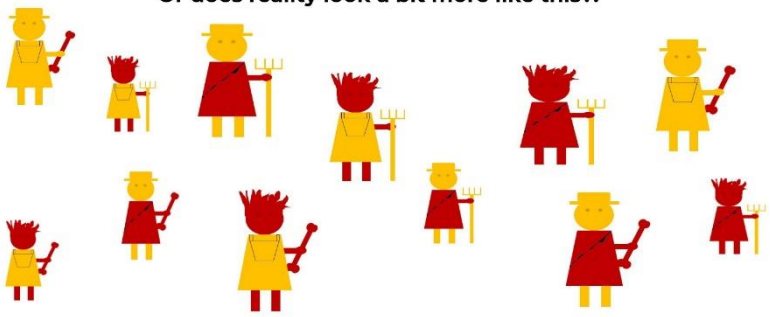
**4 million**
people in the UK are estimated to have type 2 diabetes

**£28 billion**
are the annual costs of cardiovascular disease alone to the UK health care system and the overall economy

## In recent years there has been a lively debate whether it is better to eat lots or very few carbohydrates to prevent cardiometabolic diseases




## But... Is it really that simple?? Or does reality look a bit more like this?!



## Let us find out...

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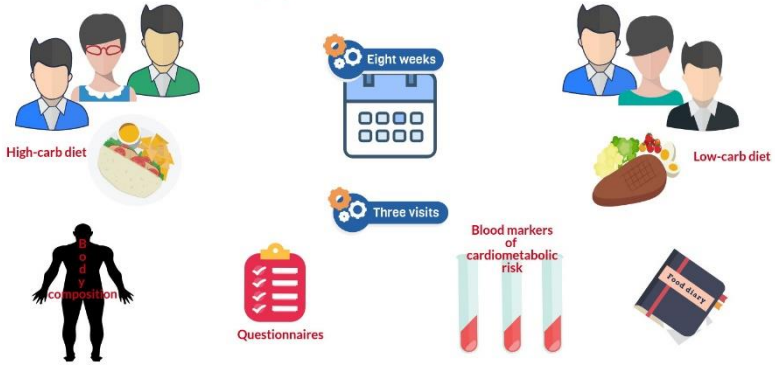
### CALIBER: Carbohydrates, lipids and biomarkers of traditional and emerging risk factors of cardiometabolic disease




The CALIBER study has been registered with ClinicalTrials.gov under reference number NCT03257085 (Researchers: Tanja Harrison and Deaglan McCullough)

The statistics provided are available at
https://www.bhf.org.uk/-/media/files/...statistics/bhf-cvd-statistics---uk-factsheet.pdf
and https://diabetes-resources-production.s3-eu-west-1.amazonaws.com/diabetes-storage/migration/pdf/DiabetesUK_Facts_Stats_Oct16.pdf